Investigating Micropollutant Removal in Sustainable Biological Wastewater Treatment Systems

Oladapo Komolafe

A thesis submitted to Newcastle University in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy

School of Engineering
Newcastle University
Newcastle upon Tyne
NE1 7RU

June, 2018

Abstract

Annually, over 300 million tonnes of chemicals used globally from human activities find their way into the aquatic environment via poorly treated wastewater in high income countries (HICs) and partially/untreated wastewater in low-middle income countries (LMICs). Some of these chemicals have been shown to have significant adverse effects on wildlife and potentially humans, hence, legislative environmental quality standards are being considered in the EU and other developed countries. This issue comes at a time when increasing energy costs are driving water companies towards sustainable treatment options – which would run counter to the use of energy intensive tertiary treatment systems that have been advocated for effective micropollutant removal. Hence, this project looked into the effectiveness of low-energy systems such as up-flow anaerobic sludge blanket reactors (UASBs) and passive-energy waste stabilization ponds (WSPs) in comparison to the high-energy activated sludge systems to remove different classes of micropollutants including industrial chemicals (PAHs and PBDEs), personal care product (triclosan), and steroidal hormones (E1, E2, E3 and EE2).

Effective analytical methods to measure the micropollutants were developed and validated (method detection limits ranging from 0.2 – 10.8 ng/L), and used to determine the occurrence and levels of the selected contaminants in UK and Brazil municipal wastewater. The observed levels of these chemicals were similar between both countries and those reported in literature- thereby indicating the prevalence of these chemicals in both LMICs and HICs. Wastewater treatment plant studies indicated that the passive-energy WSP was more effective (89 – 99 %) in removing all the classes of chemicals when compared to the energy-intensive activated sludge system (74 – 94 %) and low-energy UASB system (88 – 93 %).

The removal of these micropollutants in WWTPs was mainly due to biodegradation and sorption, while photo-degradation (in WSP) and volatilization also contributed. The experimentally determined first-order degradation rates showed that under aerobic conditions, the degradation of the different groups of chemicals was significantly different, with estrogens degrading the fastest (0.1129 h^{-1} with activated sludge inocula) and high molecular weight PAHs the slowest (0.0033 h^{-1}); while no degradation was observed under anaerobic conditions for any of the studied contaminants. Furthermore, the predicted and measured effluent concentrations indicated that effluent from the studied WWTPs poses a risk when discharged into receiving waters- as the concentrations of some chemicals were above recommended environmental quality standards (EQS), though, river dilution might ensure compliance.

Declaration

I hereby certify that this work is my own, except otherwise acknowledged, and that it has not been submitted for fulfilment of a degree at this or any other university.

Oladapo Komolafe

Table of Contents

Table of Tables

Table of Figures

Acknowledgement

My profound gratitude goes to my supervisor Dr Russell Davenport for his remarkable supervision, scientific rigour, patience, and the opportunity to tap into his wealth of knowledge and research experience. I would also like to thank my secondary supervisors, Dr Jan Dolfing, Dr Wojciech Mrozik and Dr Paola Meynet for their insightful ideas, productive discussions and contribution to this work.

Special thanks to Dr Cesar Mota, Thiago Bressani, Lucas Vassalle and other team members of the Environmental Engineering Department at UFMG, Brazil for their support and assistance during my field and laboratory work in Brazil. Thanks to the technicians within the School of Engineering for their technical support throughout my research; especially Bernard Bowler, Paul Donohoe during my extensive method development work, and Dan Curtis for chemical and microbial analysis. I would also like to acknowledge my colleagues and friends (Kishor, Marcos, Amelie, Aizat, Charles, Chamberlain, Taiwo, Tolu, Impact, Carolina, Tamara, Yemi); thanks for your valuable advice, support and friendship.

A whole-hearted thanks to my amazing parents (Mr and Mrs Komolafe), and my siblings (Cesar, Temi & Ife) for their prayers, motivation and unflinching support (financially and emotionally).

Finally, my appreciation goes to my sponsors- Engineering and Physical Science Research Council (EPSRC- reference no: EP/I025782/1), Petroleum Technology Development Fund PTDF, Nigeria and the Stapley Educational Trust.

Chapter 1: General Introduction

Chapter 1 General Introduction

Chemical pollution of water bodies has become a global issue as over one-third of the accessible freshwater in the world is used for agricultural, industrial and domestics purposes, and these activities leads to the contamination of water bodies with various industrial chemicals and consumer products (Schwarzenbach *et al.*, 2006), which are implicated in human fatalities (Figure 1-1). Over 300 million tonnes of chemical compounds are used annually as industrial chemicals and domestic products, and the cocktail mix of these spent chemicals enters into wastewater treatment plants in developed countries, where they are poorly removed and discharged into water bodies at low concentrations (ng/L to µg/L) (Schwarzenbach *et al.*, 2006). The issue is even worse in low-middle income countries (LMICs) with little or no wastewater treatment infrastructure, and as such these chemicals are deposited directly into receiving waters. Manufacturing companies are increasingly moving their operations from high income countries (HICs) to LMICs to cut-down costs, and this consequently increases the deposition of untreated/inadequately treated wastewater into the aquatic environment (Weiss *et al.*, 2016).

The ubiquitous presence of these micropollutants in the aquatic environment over the past 35 years has attracted the attention of the scientific community and government agencies. However, the issue of the adverse effects associated with chemical usage has been around for much longer. For example, antimicrobials were used to promote growth of farm animals in the 1950, before antimicrobial resistance became an issue of concern in the UK in the 1960s; eventually leading to the ban of four antimicrobial growth promoters in the EU in 1998 (Harremoës *et al.*, 2001). Another historic example, was the ban in the use of dibromochloropropane (DBCP- a pesticide used in the 1960s) in the US in 1985 after adverse reproductive effects on humans was discovered (Gee *et al.*, 2013). Today, the impact of chemicals on the environment and potentially human health has been established with many international regulations on potentially hazardous chemicals such as the Stockholm Convention on Persistent Organic Pollutants (POPs) and REACH coming into play. The EU identified in the water framework directive (WFD), and set environmental quality standards (EQS) that must not be exceeded in freshwater for 45 priority pollutants while also putting 15 other compounds on the watch list (EU, 2012; EU, 2013a) . The directive mandates member states to establish monitoring programs until end of 2018 with view of ensuring compliance to the EQS by 2021.

With the increasing cost of fuel and electricity, conventional wastewater treatment technologies are becoming more expensive (Haarmeyer, 2011), and this micropollutant removal challenge comes at a time when the water industry needs to turn to cheaper and sustainable treatment options. In fact, water treatment accounts for 3 % of the total electricity use in the UK, with wastewater treatment using half of this operational energy (Howe, 2009; Water-UK, 2017). Wastewater treatment has also been reported to account for between 3 – 5 % of the total electricity load in other developed countries (McCarty *et al.*, 2011). Furthermore, legislation in the EU has got more stringent on removal of nutrients and priority chemicals (WFD, 2000), which means that additional treatment will be required; thereby further increasing energy use. Advanced treatment technologies such adsorption onto granular activated carbon (GAC) and advanced oxidation processes (AOP) that have been suggested for the complete removal of these micropollutants from wastewater are currently not advocated because they are cost prohibitive and energy intensive (Baynes *et al.*, 2012; Gilbert, 2012).

Hence, with a steady rise in demand of potable water and use of industrial/domestic chemicals (Schwarzenbach *et al.*, 2006); and considering the ineffective micropollutant removal in the expensive conventional treatment plants (Baynes *et al.*, 2012; Luo *et al.*, 2014) and the high cost of advanced tertiary treatment, this study investigates and compares the limits of micropollutant removal in existing low energy systems to those expensive activated sludge systems. Knowing the limits of micropollutant removal in these low energy systems such as up-flow anaerobic sludge blanket reactors (UASBs) and waste stabilization ponds (WSPs), is the first step to develop sustainable secondary wastewater treatment technologies for the future capable of micropollutant removal.

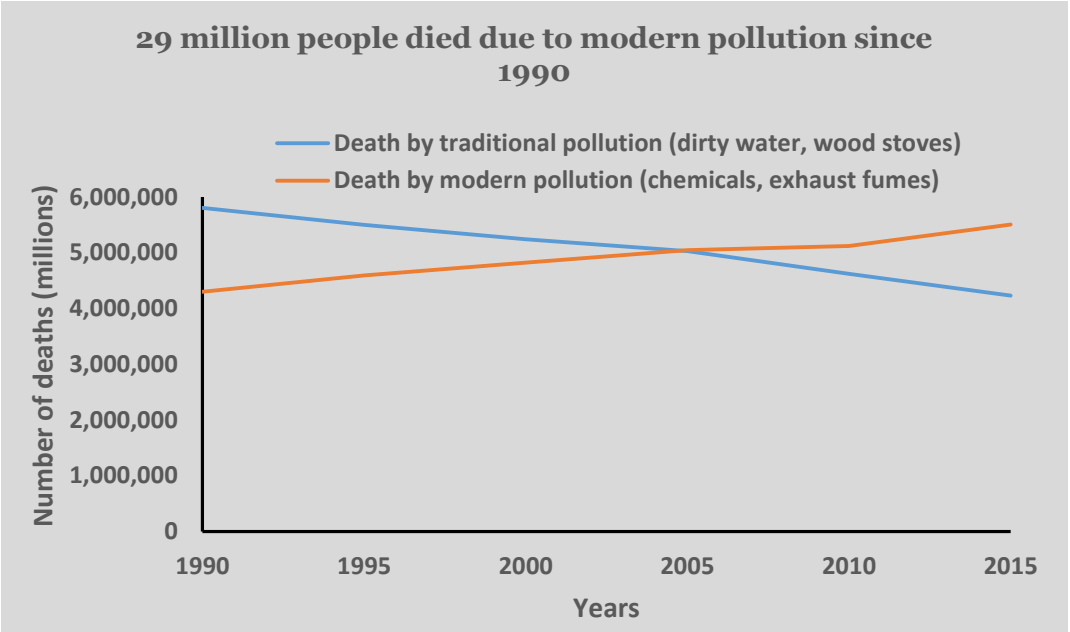


Figure 1-1 Estimated amount of death caused by pollution worldwide between 1990 and 2015 (Recreated from (Landrigan *et al.*, 2017)

1.1 Aims and objectives

The aim of this study was to investigate the limits of micropollutant removal by alternative low energy technologies (UASBs and WSPs) in comparison with conventional treatment systems (activated sludge) -towards designing sustainable micropollutant removal systems for the future.

The following objectives were pursued to achieve this aim;

1 Develop and validate analytical methods using SPE-GC-MS, SPE-GC-ECD and SPE-LC-MS to measure micropollutants including triclosan, PAHs, and PBDEs in wastewater.
2 Investigate the occurrence and removal of micropollutants (triclosan, PAHs, PBDEs) from conventional UK activated sludge WWTPs.
3 Investigate the occurrence and removal of micropollutants (triclosan, PAHs, PBDEs and estrogens) in low energy and high energy wastewater treatment systems in Brazil- considering collective system removal and the removal achieved after primary and secondary treatment individually.
4 Obtain degradation rates for the micropollutants under different redox conditions (aerobic and anaerobic) using inocula from real wastewater plants to understand degradation mechanism – aerobic (e.g. oxidative metabolism of aromatics) and

anaerobic degradation (e.g. anaerobic reductive dehalogenation for the chlorinated chemicals).

5 Investigate the relative effect of compounds structure and physio-chemical properties on their removal in biological wastewater treatment plants using different technologies.

6 Identify the putative changes in bacterial taxa associated with the degradation of the chemicals in the different inocula.

Chapter 2: Literature Review

Chapter 2 Literature review

2.1 Micropollutants and their concerns in the environment

The occurrence of micropollutants in the aquatic environment has been a major public concern around the world for decades now. These micropollutants including industrial chemicals, pesticides, pharmaceuticals, personal care products and steroidal hormones are ubiquitous in the aquatic environment at trace concentrations (sub-ng/L to µg/L) (Luo *et al.*, 2014). Even at these trace concentrations, their presence in the environment has been associated with eco-toxicity and endocrine disrupting effects including reduction in reproductive fitness and psychological disorder in aquatic wildlife (Ternes *et al.*, 2004; Baynes *et al.*, 2012). The extent of the problem is huge- there are an estimated 120,000 chemicals in use in the EU alone (ECHA, 2017). About 3000 different chemicals is used in pharmaceuticals and several thousands in personal care products (Ternes *et al.*, 2004). In the EU, over 500 million people use personal care products including skin care products, dental care products, soaps, fragrances, hair care products- amongst many others (Cosmetics-europe, 2017). Some of these chemicals have been classed as "substances with very high concern" by the European Chemicals Agency (ECHA) after decades of research into their negative effects including toxicity, carcinogenicity, mutagenicity and endocrine disruption to animals and potentially humans (ECHA, 2017). More recently, endocrine disrupting effects such as feminization and reduction in the fertility of male fish has been associated with steroidal hormones (Ternes *et al.*, 2004; Gee *et al.*, 2013). Furthermore, the effects of endocrine disruptors on humans was reviewed by (Diamanti-Kandarakis *et al.*, 2009); and the authors reported the adverse effect of endocrine disruptors on reproduction in both men and women, breast development, neuroendocrinology, thyroid, metabolism and obesity, and prostate cancer.

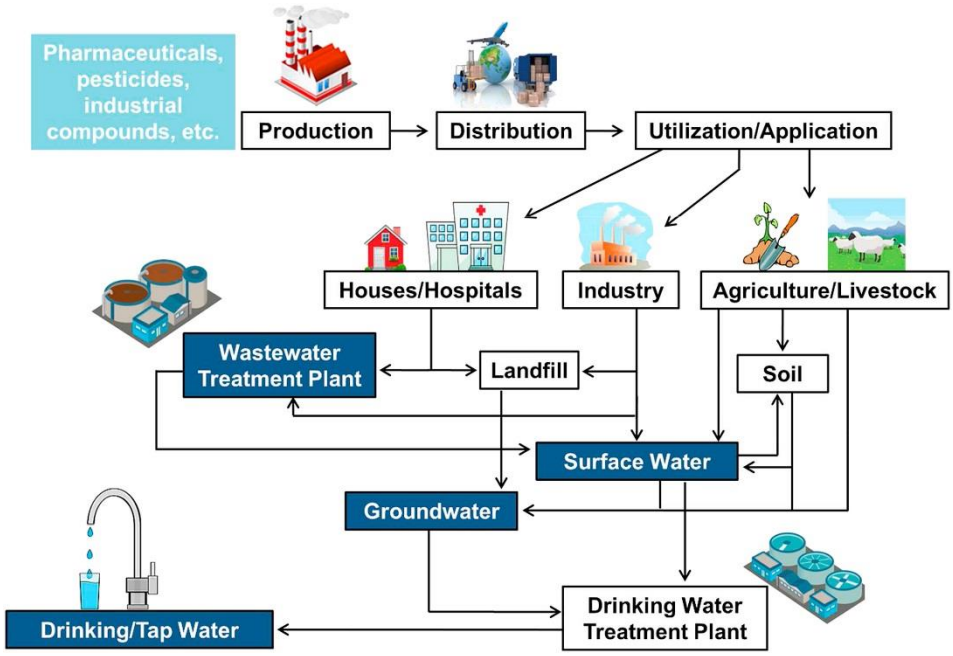


Figure 2-1 Sources, transport and fate of micropollutants in the environment (Barbosa *et al.*, 2016)

Utilization of these various chemicals and natural excretion of steroidal hormones by humans introduce them mainly into the sewage systems where they are poorly removed by wastewater treatment plants that were not specifically designed to remove them (Zhou *et al.*, 2010; Luo *et al.*, 2014). Moreover, point source contamination from the chemical manufacturing industries and run-off from roads and agricultural lands also lead to the deposition of these micropollutants into the environment (Ternes *et al.*, 2004; Wang *et al.*, 2013c). Although high percentage removal of some of the micropollutants (up to 99%) have been reported in conventional treatment plants (Luo *et al.*, 2014), the most important consideration is to achieve final effluent concentrations at recommended safety levels.

Considering the fact that the sources of some of these micropollutants cannot be eliminated, and reduction in usage of the industrial chemicals and their subsequent deposition into wastewater is unlikely at present: emphasis has been placed on enhancing wastewater treatment technologies to remove these micropollutants from the system prior to releasing the effluents into water bodies (Dytczak *et al.*, 2008). Policy and science aims to attack this issue by (a) identifying the chemicals that are most harmful, which the REACH directive addresses and feeds into the Water Framework Directive (WFD) on priority pollutants (EU, 2013a), (b) legislating on chemicals that need to be reduced to safety levels and the regulatory authorities in member states put in plans to reduce inputs- thereby pressuring water companies to implement further treatment at wastewater treatment plants.

Table 2-1 Classes and sources of micropollutants in the aquatic environment (adopted from (Luo *et al.*, 2014)

Category	Subclasses	Main sources
Pharmaceuticals	Non steroidal anti-inflammatory drugs, antibiotics, lipid regulator, and stimulants	Domestic wastewater (from excretion), hospital effluents and run off from aquaculture
Personal care products	Disinfectants, insect repellents and fragrances	Domestic wastewater (from bathing, shaving, swimming, etc)
Steroid hormones	Estrogens	Domestic wastewater (from excretion), Run-off from animal farming
Surfactants	Non-ionic surfactants	Domestic wastewater (from bathing, laundry, dishwashing)
Industrial chemicals	Flame retardants, plasticizers	Domestic wastewater (leaching out of materials, laundry, run-off from roads)
Pesticides	Insecticides, herbicides, fungicides	Domestic wastewater (cleaning run-off from gardens, roads), Agricultural run-off

2.2 Selected micropollutants of interest- rationale, sources and properties

As there are hundreds of these chemical contaminants (micropollutants) originating from different sources and everyday applications, it is impossible to study them all. Of the industrial chemicals, polyaromatic hydrocarbons- PAHs (petroleum by-products), flame retardants (polybrominated diphenyl ethers- PBDEs, hexabromocyclododecanes- HBCDD), plasticizers (bisphenol-A) and surfactants (nonylphenols, octyphenols) are among classes of chemicals of particular concern because of they are PBTs (persistent, bioaccumulative and toxic compounds), carcinogenic and endocrine disrupting (bisphenol A and nonylphenols) (EU, 2013a; ECHA, 2017). Among the personal care products, triclosan (disinfectant), galaxolide and tonalide (fragnance), benzophenone-3 (UV-screens) are of concern because of their known toxicity and endocrine disrupting effects in animals (UKTAG, 2013; Luo *et al.*, 2014). Steroidal hormones including natural estrogens (E1, E2, E3, testosterone, progesterone) and synthetic estrogen (EE2) are also an important class of high concern due to their known endocrine disruptive effects (EU, 2012; Ebele *et al.*, 2017).

Therefore, this research focused on a chemical from each class (triclosan – disinfectant, 15 PAHs and 8 PBDEs – industrial chemicals, and E1, E2, E3 and EE2 – steroidal estrogens) to cover a broad range of chemicals. Apart from the fact that they all pass through WWTPs and are ubiquitous in the environment, the rational for selecting the target compounds investigated in this study is;

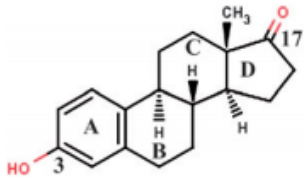
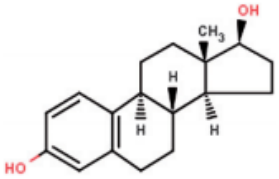
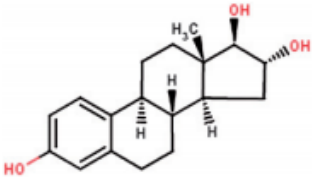
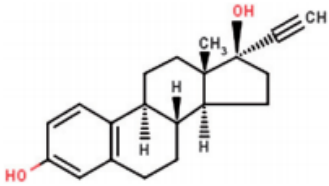
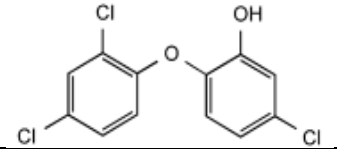
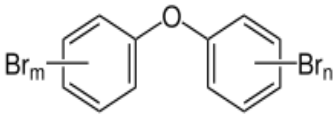
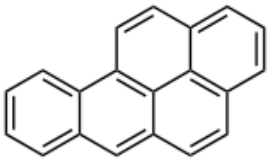
1 Little is known about their distribution and fate in low to middle income countries (LMICs)
2 They represent a wide range of compounds with different physio-chemical properties, and hydrocarbon rings with varying degrees of halogenation- which might affect their behaviour in different biological treatment systems. For example, halogenated compounds such as triclosan and PBDEs might behave differently from non-halogenated compounds (estrogens and PAHs) in wastewater treatment systems (aerobic or anaerobic). The different degree of hydrophobicity or hydrophilicity of these wide range of compounds might also lead to them behaving differently in treatment systems.
3 Public, scientific and government interest. PAHs and PBDEs were among the priority substances listed in the EU Directive 2013/39/EU (replacing 2008/105/EC) where their environmental quality standards were stipulated (EU, 2013a). Steroidal estrogens E2 and EE2 were also identified and reported in a proposal for a revised directive on priority substances EU MEMO/12/59 in 2012 (EU, 2012). The UK Technical Advisory Group on the Water Framework Directive also reported recommended environmental quality standard for triclosan in 2013 (UKTAG, 2013).

These groups of micropollutants come from different sources and their adverse effects including toxicity, bioaccumulation and endocrine disruption in humans and aquatic wildlife differ greatly (Table 2-2). Furthermore, the difference in their physio-chemical properties including their functional groups, molecular weight, octanol-water partition coefficient, water solubility amongst others, often influences their collective removal in wastewater treatment plants (Table 2-3). Additionally, the synergistic effect of a cocktail of compounds with the same or different mode of action can be more detrimental to aquatic organisms (Schwarzenbach *et al.*, 2006). Hence, it is important to investigate the removal of a diverse range of compounds in treatment plants.

Table 2-2 Classification, use and negative effects of the selected micropollutants

Micropollutant	Type	Use	Concern
Estrone (E1)	Steroid hormone (Natural)	Naturally excreted in human and livestock urine and faeces. E2 is also used as a pharmaceutical drug for hormone replacement therapy	Endocrine disruptive. Long term effect of feminization of male fish which can lead to the collapse of fish population (Coleman *et al.*, 2010; EU, 2012)
17 β-estradiol (E2)			
Estriol (E3)			
17 α-ethinylestradiol (EE2)	Steroid hormone (Synthetic)	Pharmaceutical use: is the main ingredient in contraceptive pills.	Endocrine disruptive. Long term effect of feminization of male fish which can lead to the collapse of fish population (EU, 2012).
PBDEs	Industrial chemical; BFR	Used as flame retardants in plastics, poly urethane foam and textiles.	Persistent, bioaccumulative and toxic substances; persistent; toxic to reproduction in humans and animals (Gorga *et al.*, 2013).
Triclosan	Industrial chemical	Used as an antimicrobial agent in toothpaste, soaps, cosmetics and other household products	Endocrine disruptor, toxic (Lozano *et al.*, 2013).
PAHs	Industrial Chemical	Product of incomplete combustion of organic materials, by-product in the processing of raw materials	Carcinogenic and mutagenic compounds to humans and animals (Yao *et al.*, 2012)

Table 2-3 Structure and physio-chemical properties of the selected micropollutants

Compound	Structure	Molecular weight (g/mol)	Log K_{ow}	Water solubility (mg/l)
Estrone (E1)[a,b]		270.4	3.43	13 at 20 °C
17 β-estradiol (E2)[a,b]		272.4	3.94	13 at 20 °C
Estriol (E3)[a,b]		288.4	2.81	13 at 20 °C
17 α-ethinylestradiol (EE2)[a,b]		296.4	4.15	4.8 at 20 °C
Triclosan[c]		289.5	4.80	10 at 20 °C
Polybrominated diphenyl esters (PBDEs)[d]		PeBDE = 485.8 – 564.7 OBDE = 647.3 – 801.8 DBDE = 880.4 – 959.2	PeBDE =6.57 OBDE = 6.29 DBDE = 6.27	PeBDE = 13.3 OBDE = 0.5 DBDE = <0.1 at 25 °C
Polyhydric hydrocarbons (PAHs)[e,f]	 Benzo(a)pyrene	Phe = 178.2 An = 178.2 Bp = 252.3 Ip = 276.2	Phe = 4.57 An = 4.68 Bp = 6.13 Ip = 6.4	Phe = 0.82 An = 0.093 Bp = 0.0018 Ip = 0.00053 at 25°C

A = (Racz and Goel, 2010); b = (Silva *et al.*, 2012); c = (Sabaliunas *et al.*, 2003); d = (EC, 2006); e = (De Maagd *et al.*, 1998); f = (EC, 1994).
PeBDE = Pentabromodiphenyl ether; OBDE = Octabromodiphenyl ether; DBDE = Decabromodiphenyl ether
PHE = Phenanthrene; AN = Anthacene; BP = Benzo(a)pyrene; IP = Indeno(1,2,3,-cd)pyrene

2.3 Analytical methods for the detection and quantification of these micropollutants

The occurrence of these micropollutants at trace concentrations (sub-ng/L to µg/L) in wastewater and water bodies has posed difficulties in their detection and analysis. These low concentrations are close to the limit of detection of many analytical methods - leading to intensified method development efforts by scientists around the world. The analytical process to measure these compounds includes sample collection and storage, extraction, and analysis.

Several techniques including liquid-liquid extraction (LLE), pressurized liquid extraction (PLE), solid phase extraction (SPE), solid phase micro-extraction (SPME) and stir-bar sorptive extraction (SBSE) have been employed to extract these compounds from wastewater (Alda and Barceló, 2001; Wenzl *et al.*, 2006; Labadie *et al.*, 2010; Tohidi and Cai, 2015). SPE is the most commonly used method today because of its selectivity, low solvent requirement, reproducibility and generation of extracts that does not require additional clean up (Alda and Barceló, 2001; Sánchez-Avila *et al.*, 2009). Careful selection of the adsorbent and solvent elution protocol are usually the most important part of the SPE extraction process. Octadecyl (C_{18}) bonded end-capped silica has been the most widely used SPE sorbent to extract organic compounds from water and wastewater because of its wide applicability (Alda and Barceló, 2001), although mixed mode sorbents such as the Oasis HLB are getting increasingly popular for the extraction of pharmaceuticals and personal care products as they can be used to extract a wide range of chemicals (Samaras *et al.*, 2011).

Determination of these micropollutants in environmental samples is commonly carried out with gas chromatography with mass spectrophotometry (GC-MS) and electron capture detector (GC-ECD), or by liquid chromatography (LC) coupled with a fluorescence, UV or mass spectrometric (MS) detector (Alda and Barceló, 2001; Sánchez-Brunete *et al.*, 2007). The MS is the preferred detector both in GC and LC because of its superb specificity and sensitivity, and therefore enables unequivocal identification of different compounds in complex environmental samples (Busetti *et al.*, 2006). However, the high procurement and maintenance costs associated with GC-MS or LC-MS equipment compared to GC/LC coupled with other detectors (such as UV, ECD) have prevented environmental analysis of these compounds in developing countries with less resources.

2.4 Occurrence of micropollutants in the environment and their removal by WWTPs

The selected micropollutants have been detected and reported in WWTPs around the world (Table 2-4). The concentrations of these compounds range from a few pg/L to µg/L, and vary in the influent and effluent due to the consumption rate of this chemicals in different cities, the fraction that enters the WWTPs and the removal rates in the different WWTPs (Luo *et al.*, 2014). Triclosan, PAHs and PBDEs were generally detected in relatively higher concentrations, as these chemicals are used in large quantities in various applications as mentioned above. However, the majority of wastewater goes into receiving waters untreated in LMICs especially.

The removal of these different groups of chemicals varies in biological wastewater treatment systems around the world. This difference in removal observed in the different countries may be result of the difference in the technologies applied, operation conditions, variation in efficiency and influent concentrations (Verlicchi *et al.*, 2012). Verlicchi *et al*. reviewed the fate of 118 pharmaceuticals in 264 wastewater treatment plants and observed that removal efficiencies varied due to the different physio-chemical properties of the chemicals and operational conditions of the WWTPs including aerobic, anaerobic and anoxic reactors, solid retention time, pH and water temperature (Verlicchi *et al.*, 2012). The detection of these chemicals in treated effluents also shows the ineffectiveness of the treatment plants to remove them. The *predicted no-effect concentration* (PNEC) values of E1, E2, E3 and EE2 to aquatic organisms was determined to be 6, 2, 60 and 0.1 ng/L respectively (Caldwell *et al.*, 2012), while that of triclosan is 100 ng/L (UKTAG, 2013). The European Commission also set the maximum allowance concentration and environmental quality standard (MAC-EQS) for six PAHs including naphthalene, anthracene, fluoranthene, benzo(a)pyrene, benzo(b)fluoranthene and benzo(ghi)perylene to be 130,000 ng/L (13 µg/L), 100 ng/L, 120 ng/L, 270 ng/L, 17 ng/L and 0.82 ng/L respectively; and sum of six PBDE congeners including BDE 28, 47, 99, 100, 153 and 154 as 140 ng/L (EU, 2013a). The concentrations of these chemicals in effluent of treatment plants around the world often exceed these safety levels, and therefore poses threat to aquatic wildlife. However, this is dependent on dilution by the receiving water body, which might ensure compliance to this safety levels under certain circumstances.

Table 2-4 The concentrations and removal of the selected compounds in WWTPs around the world

Selected compounds	Countries	Influent (µg/L)	Effluent (µg/L)	Removal (%)	References
E1	China, Germany, France, US, Brazil, Australia	0.01 – 0.17	<0.001 – 0.08	75 – 91	1, 2
E2	China, Germany, France, US, Brazil	0.002 – 0.05	<0.001 – 0.007	93 – 100	1, 2
E3	China, Korea, France	0.125 – 0.80	ND – 0.03	90 – 100	1, 3
EE2	China, Germany, Italy, France, US, Brazil	0.001 – 0.003	<0.001 – 0.007	44 – 100	1, 2
Triclosan	China, UK, US, France, Australia	0.03 – 23.9	0.01 – 6.88	71 – 99	1, 4
PAHs	China, Italy, Ireland	0.7 - 5.8	0.2- 2.2	67- 85	5, 6, 7
PBDEs	Canada, US, China, UK, Australia, Italy	0.26 – 4.3	0.04 – 0.9	79 - 86	5, 8

1 = (Luo *et al.*, 2014); 2 = (Pessoa *et al.*, 2014); 3 = (Gabet-Giraud *et al.*, 2010); 4 = (Kookana *et al.*, 2011); 5 = (Wang *et al.*, 2013c); 6 = (Fatone *et al.*, 2011), 7 = (Jones *et al.*, 2012); 8 = (Ratola *et al.*, 2012). ND = not detected

2.5 Micropollutant removal mechanisms

Micropollutants are removed in WWTPs by physical, chemical and biological processes. Among these processes, sorption and biodegradation are the main removal mechanisms, with volatilization only playing a minor role (Liu *et al.*, 2009; Verlicchi *et al.*, 2012). Removal by abiotic processes (physical and chemical processes) includes adsorption, volatilization and photodegradation, while biotic processes (biological processes) involve biodegradation.

Micropollutants are removed in WWTPs by sorption onto sludge during primary and secondary treatment. This occurs by either adsorption on the liquid fraction of the sludge, hydrophobic interactions between the aliphatic and aromatic groups of a compound and the lipophilic cell membrane of microorganisms or adsorption via electrostatic interactions between some positively charged compounds and negatively charged surface (microorganism, clays, humic substances etc.) (Karathanasis and Johnson, 2003; Ternes *et al.*, 2004). The physio-chemical properties of different compounds such as polarity, solubility and hydrophobicity govern their adsorption removal rates, as well as the WWTPs operating conditions (i.e. hydraulic retention times (HRT), pH) (Lofrano, 2012). For example, highly hydrophobic compounds with high octanol-water partition coefficient (Log K_{ow}) such as

PBDEs would sorb better to suspended solids than more water soluble estrogens with a lower Log K_{ow} (Table 2-3). The sorption potential of a compound is indicated by their Log K_{ow} value. A Log $K_{ow} <$ 2.5 indicates low sorption potential, 2.5 < Log K_{ow} < 4 indicates medium sorption potential, and Log $K_{ow} >$ 4 indicates high sorption potential (Rogers, 1996). Adsorption potential (k_d) of a compound, is reported be higher in secondary sludge (20 g/L of suspended solids (SS) than primary sludge (2 g/L of SS) as microorganisms constitute more of the SS content- hence, compounds sorb more during secondary treatment (Ternes *et al.*, 2004). For example, it has been suggested that between 80 – 90 % of triclosan is removed by adsorption in conventional WWTPs (Chen *et al.*, 2011; Lozano *et al.*, 2013). Furthermore, about 80 % for PAHs and PBDEs were reported to be partitioned onto the solid particulate matter, and removed by sorption in real WWTPs (Sánchez-Avila *et al.*, 2009).

The volatilization tendency of compounds is characterised by their Henry's law constant (K_H) such that compounds with K_H values between 10^{-2} to 10^{-3} mol/(m^3.Pa) will tend to volatilize during treatment (Stenstrom *et al.*, 1989). For example, low molecular weight PAHs (i.e. naphthalene) with a K_H value of 0.02 mol/(m^3.Pa) will most likely volatilize during treatment (De Maagd *et al.*, 1998). In fact, aeration during the activated sludge treatment can further intensify the volatilization process (Luo *et al.*, 2014). Photodegradation aided by sunlight has been suggested as a removal mechanism for micropollutants (Coleman *et al.*, 2010). Photodegradation of micropollutants occurs either by direct photolysis - where direct absorption of lights leads to the degradation of compounds or by photosensitization - where substances in the matrix such as humic compounds absorbs light and transfers this energy to degrade the compounds (Sornalingam *et al.*, 2016). In fact, previous studies have reported photodegradation of estrogens under visible light (Sornalingam *et al.*, 2016), UV light and sunlight, triclosan under UV and sunlight (Buth *et al.*, 2010; Tamura and Yamamoto, 2012), PAHs under mimicked sunlight (Saeed *et al.*, 2011), and PBDEs under UV, mercury, xenon and sunlight (Pan *et al.*, 2016).

Biodegradation of micropollutants mostly occurs during secondary treatment leading to partial degradation (formation of by-products) or complete mineralization of the compounds. The mechanisms of biodegradation in treatment systems is either by cometabolism - where the bacteria partially or completely decomposes the micropollutants without using them as an energy source or mixed substrate growth occurs - where the micropollutants are used as a carbon and energy source by the bacteria (Ternes *et al.*, 2004). In treatment plants, the hydraulic retention time and sludge age influences their micropollutant removal (Ternes *et al.*, 2004; Alvarino *et al.*, 2014). As the sludge age increases, the bacterial abundance increases

and becomes more diverse, and therefore are more capable in degradation micropollutants - a sludge retention time of between 12 – 15 days in activated sludge plants has been reported to improve removal of estrogens and pharmaceuticals (Ternes *et al.*, 2004; Suarez *et al.*, 2010). The chemical structure of micropollutants also influences their biodegradability. For example, compounds with long and highly branched chains, polycyclic compounds and compounds characterized by functional groups such as halogens or sulfate are often harder to degrade (Tadkaew *et al.*, 2011; Luo *et al.*, 2014).

2.6 Biodegradation of micropollutants under different redox conditions

The biodegradation of micropollutants also varies with different redox conditions such as aerobic (molecular oxygen present), anoxic (molecular oxygen absent but nitrate present) and anaerobic conditions (molecular oxygen and nitrate absent) (Ternes *et al.*, 2004; Chen *et al.*, 2011). During aerobic catabolism of aromatic compounds, oxygen acts as the final electron acceptor and co-substrate for the hydroxylation and oxygenolytic ring cleavage, while anaerobic catabolism proceeds by reductive reactions (Figure 2-2) (Ghosal *et al.*, 2016). Since wastewater treatment technologies such as activated sludge and up-flow anaerobic sludge blanket reactors treat wastewater under aerobic and anaerobic conditions respectively, understanding the fate of these compounds under both conditions is important and has been studied in literature. However, most studies have focused on aerobic conditions because of the popularity of the activated sludge systems. According to Alvarino *et al*. biodegradation of estrogens (E1, E2 and EE2) was higher under aerobic conditions (62 – 95%) than anaerobic conditions (38 – 60 %) in their lab scale continuous activated sludge and UASB reactors (Alvarino *et al.*, 2014). Rapid transformation and high removal rate of E3, E2, E1 and EE2 has been reported to occur under nitrifying conditions with activated sludge and ammonia oxidizing bacteria (Haiyan *et al.*, 2007; Dytczak *et al.*, 2008; Gaulke *et al.*, 2008).

Furthermore, Chen *et al*. reported degradation of triclosan under aerobic conditions with activated sludge in their batch studies, but observed resistance to degradation under anoxic and anaerobic conditions (Chen *et al.*, 2011). There is limited information on degradation of PAHs in wastewater treatment systems under different redox conditions, and when available they have often been on sludge treatment or soil remediation rather than the main wastewater treatment stream. Trably *et al*. compared removal of PAHs during aerobic and anaerobic sludge treatment, and reported relatively higher removal efficiencies (up to 90 % for low molecular weight PAHs and 50 % for higher molecular weight PAHs) under aerobic conditions. They also reported that the lighter PAHs were removed to a lower extent in

comparison to the heaver ones (Trably *et al.*, 2005). It has also been reported that bacteria generally favour aerobic degradation of PAHs over the other redox conditions (Ghosal *et al.*, 2016). Biodegradation of PBDEs has also been reported under aerobic and anaerobic conditions. Stiborova *et al*. reported about 62 – 78 % degradation of eight PBDE congeners by activated sludge under aerobic conditions (Stiborova *et al.*, 2015). There has also been reports of anaerobic degradation and debromination of PBDEs in some studies (He *et al.*, 2006; Xia, 2013). However, PBDEs degradation is slower under anaerobic conditions (Gerecke *et al.*, 2005). Anaerobic reductive dehalogenation has been suggested to be an important biodegradation mechanism for halogenated compounds under methanogenic and sulphate reducing conditions. Chemicals such as PBDEs and PCBs, have been reported to undergo anaerobic dehalogenation during wastewater treatment where highly halogenated congeners are converted less-halogenated congeners thereby increasing their biodegradability (Xia, 2013; Yao *et al.*, 2014; Expertise, 2015). Reductive dechlorination of triclosan has been reported by bacteria during anaerobic digestion of sludge (Ogunyoku and Young, 2014; Smith *et al.*, 2015b), and by algae strains (Wang *et al.*, 2013b). Some reported degradation rates of all the classes of chemicals under aerobic conditions in literature are shown in Chapter 4 & Chapter 5.

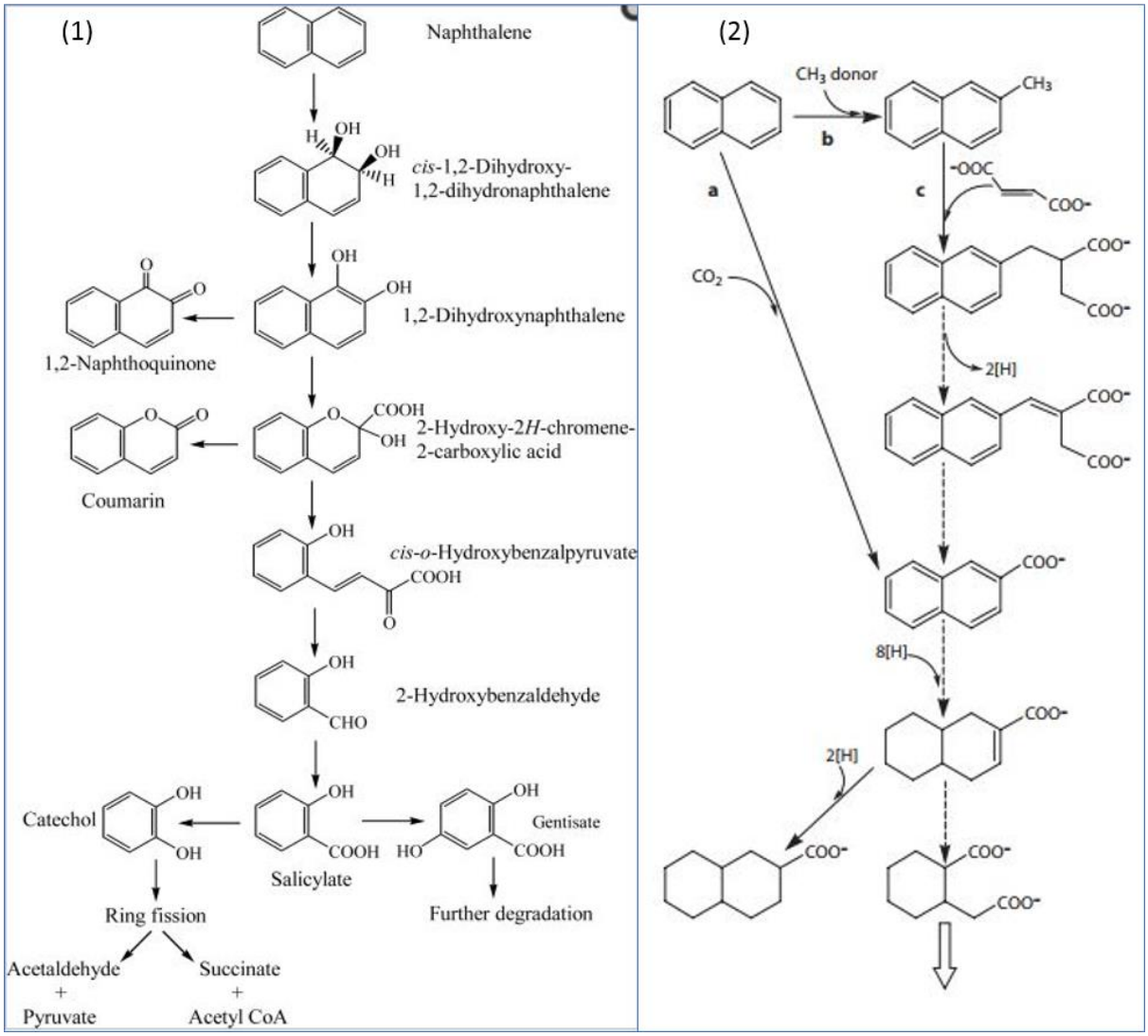


Figure 2-2 Degradation pathway of naphthalene by bacteria under (1) aerobic conditions (Seo *et al.*, 2009), and (2) anaerobic conditions (Foght, 2008)

In summary, the degradation rates of these chemicals were faster under aerobic conditions compared to anoxic and anaerobic conditions. However, most of the studies either used activated sludge inocula under different redox conditions or tried to acclimatize inocula from river or soil sediments to anaerobic conditions. Furthermore, estrogens degraded within a few hours in literature while PBDEs took several days to degrade. This indicates the effect of the chemical structure of different groups of compounds on their degradation. The importance of rates for all process is that they can be used to calculate the removal of any chemical for a given influent concentration and hydraulic retention time (i.e. flow and volume of the reactor) (Levenspiel, 1999)- which can then determine the true limits of engineering systems for micropollutant removal.

2.7 Sustainability issues of current WWTPs

In this age of increasing energy costs, wastewater treatment in most developed countries have become unsustainable ; and this is even a bigger burden in low-middle income countries with less resources (Liu *et al.*, 2004; Logan, 2005; Heidrich *et al.*, 2010; McCarty *et al.*, 2011). In the UK, wastewater treatment uses 1.5 % of the total electricity consumption and accounts for up to 0.5 % of the total CO_2 emission (Water-UK, 2017). The UK government is committed to reducing the total CO_2 emission by 80 % compared to 1990 levels, and produce 15 % of the total energy requirement from renewable energy by 2020- for which the water companies have agreed to a 20 % renewable energy generation by 2020 (Howe, 2009). With this upcoming new legislations on the removal of micropollutants by the WFD, and the pressure on the water companies by member states, adding proposed tertiary treatment systems will further increase energy use- thereby making the reduced energy and emissions target unachievable. These advanced treatment systems such as adsorption with granulated activated carbon and advanced oxidation process will increase wastewater treatment energy demand by 30 % and would cost between £26 - 30 billion to equip over 1,300 WWTPs in the UK (Gilbert, 2012). Hence, the need to look at alternative technologies, such as anaerobic treatment technologies or passive systems (waste stabilization ponds) that have the potential for reducing wastewater treatment costs (no aeration requirements) and perhaps potential for effective micropollutant removal.

2.8 Microbial degradation (biodegradation) of micropollutants

Microbial degradation is the most important process of micropollutants removal from the environment as it can result to complete mineralization of the chemicals in natural and engineered eco-systems (Rücker and Kümmerer, 2012). Intrinsically, the biodegradability of a chemical depends on several factors such as physio-chemical properties of the chemical, environmental conditions (pH, redox conditions), presence and activity of degrading microbial taxa, etc. (Luo *et al.*, 2014). The presence and activity of specific degraders among the microbial community present in biological systems will most likely determine the probability and extent of chemical degradation (Martin *et al.*, 2017). Several bacteria genera have been reported to degrade aromatic compounds, especially under aerobic conditions (Peng *et al.*, 2008; Ghosal *et al.*, 2016). For example, *Pseudomonas* and *Rhodococcus* have been identified has versatile degraders of chemical pollutants and have been associated with the degradation of triclosan (Lee and Chu, 2013), PAHs (Peng *et al.*, 2008; Ghosal *et al.*, 2016), estrogens (Yoshimoto *et al.*, 2004; Yu *et al.*, 2013) and PBDEs (Robrock *et al.*, 2009). However, little is known about the bacteria taxa responsible for the degradation of these

chemicals during wastewater treatment and their degradation pathways. Hence, this study explores the identification of bacteria taxa in the microbial inocula responsible for degradation of triclosan, PAHs, estrogens and PBDEs.

2.9 Research gaps

The following research gaps have been identified;

1. Little is known about the occurrence of these classes of micropollutants in wastewater streams of low-middle income countries (LMICs) despite these chemicals being used in similar quantities to high income countries (HICs).
2. Little is also known about removal and fate of these classes of micropollutants in low energy treatment systems including UASBs and WSPs. When this information is available, there is little information on which part of these treatment plants the compounds were removed and to what extent.
3. There is little or no information comparing degradation rates of these compounds under aerobic and anaerobic conditions, especially for triclosan, PAHs and PBDEs.
4. There is lack of knowledge on the removal of these chemicals via photodegradation in WSPs.

Chapter 3: Method Development and Validation

Chapter 3 Method development and Validation

3.1 Quantification of triclosan and polyaromatic hydrocarbons (PAHs) in wastewater by gas chromatography with mass spectrophotometry (GC-MS)

3.1.1 Introduction

Triclosan and PAHs are important organic contaminants because they are ubiquitous and persistent in the environment (Thompson *et al.*, 2005; Wang *et al.*, 2009). PAHs enter the aquatic environment mainly via anthropogenic sources, including discharge of petroleum and petroleum- related products and via urban storm water carrying PAHs from asphalt and car exhaust particles (Qi *et al.*, 2013; Wang *et al.*, 2013c). Sixteen PAHs were included in the EU and US lists of priority pollutants because of their recalcitrance, suspected carcinogenicity and mutagenicity (Cao *et al.*, 2005; Jones *et al.*, 2012; Yao *et al.*, 2012). These PAHs are: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, antracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-cd)pyrene (Wenzl *et al.*, 2006). 7 of them have been included as priority substances by the EU and have EQS (Environmental Quality Standard) values associated with them- i.e. they can potentially cause harm to aquatic wildlife (EU, 2013a)

Triclosan is a commonly used antimicrobial agent in personal care products (including hand wash, body soaps, toothpastes, mouthwashes etc.); as such, it enters the aquatic environment via discharge from households into domestic sewage (Zhao *et al.*, 2010). These personal care products typically contain about 0.1 – 0.3 % (w/w) of triclosan (Sabaliunas *et al.*, 2003; Fiss *et al.*, 2007); global production exceeded 1500 t in 2002, of which 350 t was used in Europe (Singer *et al.*, 2002). Triclosan is a bioaccumulative and persistent compound and its occurrence in the aquatic environment has been of growing concern due to its toxicity to aquatic organisms including algae, daphnids and fishes (Singer *et al.*, 2002; Ying and Kookana, 2007; Kookana *et al.*, 2011). This has led the UK Technical Advisory Group on the Water Framework Directive to provide recommendations on environmental standards of triclosan in surface waters (UKTAG, 2013).

The occurrence of triclosan and PAHs in wastewater (in ng/L or µg/L) and their fate during wastewater treatment has been extensively reported in the literature. The majority of these studies focused only on the occurrence of these compounds in the aqueous phase without due consideration for the large proportion that partitioned onto the particulate matter. Due to

incomplete removal by the wastewater treatment plants, these compounds are discharged to water bodies, in dissolved phase or adsorbing to particulate matter (Qi *et al.*, 2013). Since, this is a major conduit of PAH and triclosan into the aquatic environment, it is imperative to adopt an effective analytical methodology to quantify the presence of these compounds in both the dissolved and particulate matter phase of wastewater.

Several techniques including liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE) have been employed to extract triclosan and PAHs from water and wastewater (Wenzl *et al.*, 2006; Tohidi and Cai, 2015). LLE using non-polar solvents and SPE are the most commonly used analytical method for extracting the studied compounds (Bester, 2005; Busetti *et al.*, 2006; Brum and Netto, 2009; Tohidi and Cai, 2015), with the latter getting increasingly popular because of its selectivity, reproducibility and low solvent requirement (Busetti *et al.*, 2006; Moja and Mtunzi, 2013). Determination of triclosan and PAHs in environmental samples is commonly carried out by gas chromatography coupled with mass spectrophotometry (GC-MS), or by liquid chromatography coupled with a fluorescence (only PAHs), UV or mass spectrometric (MS) detector (Bester, 2005; Cao *et al.*, 2005; Busetti *et al.*, 2006; Kookana *et al.*, 2011). The MS detector is to be preferred as it offers better specificity and sensitivity (Busetti *et al.*, 2006; Sánchez-Brunete *et al.*, 2007), which is particularly pertinent to identifying target compounds in difficult complex environmental samples such as wastewater. Furthermore, GC-MS combined with stable isotope quantification ensures unequivocal identification of PAHs and triclosan in environmental samples (Cao *et al.*, 2005).

Optimization of the SPE based extraction method and chromatographic separation is essential for better detection limits and recoveries of the compounds especially the 15 PAHs with a wide molecular weight and Log K_{ow} range. Unlike most other SPE-GC-MS based methods, this approach enables the reliable determination of the analytes in the aqueous and particulate matter phase simultaneously; thereby eliminating the need for a separate analyte quantification in solids and as a result saving essential resources. Furthermore, a robust and reliable method that simultaneously measures the analytes in both phases (aqueous and particulate matter) is essential to our planned biodegradation studies in bio-solids.

In this study, methods using solid phase extraction and GC-MS were optimized and validated for the determination of triclosan and the 15 priority PAHs in the aqueous and particulate matter phase of wastewater. The study also compared the effect of pH sample adjustment and SPE sorbent amount on the extraction efficiency of triclosan and PAHs from wastewater.

Analytical performance was evaluated based on detection limits, linear working range and repeatability. The methods were then applied to evaluate the removal of triclosan and PAHs in municipal wastewater treatment plants in North East England.

Table 3-1 Names, abbreviations, physical and chemical properties of the triclosan and the 15 PAHs investigated in this study

Compound	Acronym	Molecular formula	Log K_{ow}, [c]	Adsorption potential [b]	Solubility (mg/L) at [d]
Triclosan	TCS	$C_{12}H_7Cl_3O$	4.8[a]	High	10.0[a]
Naphthalene	Naph	$C_{10}H_8$	3.30[c]	Medium	31.0
Acenaphthylene	Acy	$C_{12}H_8$	3.94	Medium	16.1
Acenaphthene	Ace	$C_{12}H_{10}$	3.92	Medium	3.8
Fluorene	Flu	$C_{13}H_{10}$	4.18	High	1.9
Phenanthrene	Phen	$C_{14}H_{10}$	4.46	High	1.1
Anthracene	Anth	$C_{14}H_{10}$	4.45	High	0.062
Fluoranthene	Flt	$C_{16}H_{10}$	5.16	High	0.240
Pyrene	Pyr	$C_{16}H_{10}$	4.88	High	0.132
Benz(a) anthracene	BaA	$C_{18}H_{12}$	5.66	High	0.011
Chrysene	Chry	$C_{18}H_{12}$	5.81	High	0.0019
Benzo(b) fluoranthene	BbF	$C_{20}H_{12}$	5.78	High	0.0015
Benzo(a)pyrene	BaP	$C_{20}H_{12}$	6.13	High	0.0038
Indeno (1,2,3-cd)pyrene	InPy	$C_{22}H_{12}$	6.70	High	0.00019
Dibenz(a,h) anthracene	DiahA	$C_{22}H_{12}$	6.75	High	0.00056
Benzo(ghi) perylene	BghiP	$C_{22}H_{12}$	6.63	High	0.00083

[a] *adopted from (Kantiani et al., 2008)*

[b] *When Log K_{ow} < 2.5, adsorption potential is low, medium when >2.5 but < 4, high when > 4 (Rogers, 1996)*

[c] *adopted from (Sánchez-Avila et al., 2009)*

[d] *solubility at 25 °C. Adopted from (Mackay et al., 2006)*

3.1.2 Experimental

3.1.2.1 Materials and reagents

A certified standard solution of 15 mixed priority PAHs (including naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, chrysene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene and benzo(*ghi*)perylene at 2 mg/ml in dichloromethane) and triclosan (100 mg) were purchased from Sigma, (UK). Isotope labelled $^{13}C_{12}$-Triclosan (50 µg/ml in methanol) and a mix of deuterated PAHs (including acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12} at 2 mg/ml in dichloromethane) were purchased from Wellington Laboratories, (Canada, via Greyhound Chromatography, UK) and Accustandard (via Kinesis UK) respectively. Internal standards meclofenamic acid (MFA, 100 mg) and p-Terphenyl-d_{14} (100 mg) were also purchased from Sigma Aldrich and Greyhound Chromatography (UK) respectively. Derivatization reagent BSTFA with 1% TCMS was also purchased from Sigma Aldrich (UK).

Stock solutions were prepared by dissolving the reference and surrogate standards in methanol or acetone at various concentrations (1000 µg/ml for triclosan, 20 µg/ml for $^{13}C_{12}$-triclosan, 20 µg/ml for PAHs, and deuterated PAHs). Working solutions were then prepared by diluting the stock solutions in acetone or methanol and dichloromethane for sample fortification and instrumental analysis respectively. All solutions were stored at 4 ^{o}C and allowed to reach room temperature for 15 mins before use. Ultra-trace grade of methanol, acetone, dichloromethane and isopropanol were obtained from Sigma Aldrich (UK). Cartridges used for solid phase extraction were Isolute C_{18} (1000mg, 6 ml) and Isolute C_{18} (500mg, 6 ml), and were purchased from Biotage (UK). Glass microfiber filters were purchased from Sartorius (MGB filters, 0.7mm thick, 1.0 µm particle retention).

3.1.2.2 Sample collection

Grab samples of wastewater (raw influent and final effluent) were collected from two nitrifying activated sludge plants treating municipal wastewater in North East England. The population equivalents of WWTP A and WWTP B are 22,500 and 28,800 respectively. Samples were collected in cleaned and disinfected (with 1% Virkron for 24 hours, followed by several rinse cycles with distilled water) high density polyethylene (HDPE) containers - analysis of container wash water demonstrated that they were uncontaminated with the target compounds. Samples were stored at 4 ^{o}C upon arrival in a cold room and were used within 24 hours. Samples were percolated directly through the SPE cartridges to allow quantification of

the chemicals in the combined aqueous and particulate phase (i.e. total concentration). An aliquot of the same samples were filtered through glass microfiber filter to estimate the concentration of the chemicals in the aqueous phase alone (Sartorius MGB filters, 0.7mm thick, 1.0 µm particle retention), before further processing, using solid phase extraction (SPE). The concentration of chemicals in the particulate phase were determined by subtracting their concentration in the aqueous phase from the total concentration. Effluent from WWTP A was used for the method development. PAH was surveyed only in WWTP A, while triclosan was investigated in both plants.

3.1.2.3 GC instrumentation

Analysis was performed on an Agilent 7890A gas chromatography system equipped with an Agilent 5957C Mass Spectrometer and an Agilent 7683B automatic injector. A fused silica capillary column (DB-5MS; 60m x 0.25mm I.D. x 0.25µm film thickness) from J & W Scientific, USA was used for chromatographic separation for both triclosan and PAHs analysis. The inlet was fitted with an SGE single taper deactivated glass liner and samples were injected (1 µl) in pulsed splitless mode (1 min. splitless, then 30 ml/min split). The inlet and interface temperature was set at 280 ^{o}C and 310 °C respectively. Heluim (99.999 %) was used as carrier gas in constant flow mode (flow-rate 1 ml/min, initial pressure of 120 kPa, split at 30 ml/min, velocity 91.3 cm sec^{-1}). The GC was temperature programmed from 50 - 310 °C; held at 50 °C for 2 min then increased to 310 °C at 5 °C /min, then held at 310 °C for 21 min for PAH analysis while GC program for triclosan analysis was from 50 - 310 °C; held at 50 °C for 1 min then increased to 310 °C at 10 °C /min, then finally held at 310 °C for 10 min. Total run time was 75 min and 35 min for PAH and triclosan analysis respectively. The Mass Spectrometer was operated in electron-impact ionization (EI) mode with electron voltage set at 70 eV, source temperature at 230 °C, quad temperature at 150 °C, and multiplier voltage at 1800 V. Acquisition was initially performed in scan mode (50 – 600 amu/sec), but mainly in Selected Ion Monitoring (SIM- 58 ions, 0.7 cps, 20 ms dwell) mode to improve sensitivity and selectivity. Data was acquired and processed using a Chemstation Chromatographic Data System (version. 8.3). The ion corresponding to the fragmentation of each analyte was used for quantification and confirmation (Table 3-2). Peaks were identified and labelled after comparison of their mass spectra with those of the NIST05 library if > 90% fit or from their elution order in the literature (Qiao *et al.,* 2013). Triclosan and PAHs were analysed separately, but with a similar instrumentation method that differed only by the GC temperature program applied. Poor sensitivity of PAHs was observed at concentration below 20 ng/L, especially for the high molecular weight PAHs, due to high end discrimination.

Hence, different GC injection liners (straight liner, green liner and blue liner) were tested to improve the sensitivity of the GC-MS. The GC-MS was vented and ion source was cleaned before installing the capillary column. After 2 hours, the mass spectrophotometer was tuned using the 5975 Auto-tune program- this uses perfluorotributylamine (PFTBA- a tuning and calibration mixture) and focuses on m/z 69, 219 and 502 to system optimization.

Table 3-2 Characterization of triclosan, 15 PAHs, relative surrogates and internal standards by GC-MS

Compound(s)	Molecular weight category	Retention time (min.)	Characteristic ion (m/z)
	Triclosan		
Triclosan-TMS*	-	17.34	345, 347, 360, 362, 200
$^{13}C_{12}$-Triclosan-TMS (surrogate)	-	17.34	357, 372, 374
MFA-TMS (Internal standard)	-	19.50	242, 244, 367
	PAHs		
Naphthalene	LMW (2 rings)	21.621	128
Acenaphthylene	LMW	29.091	152
Acenaphthene	LMW	29.932	153
Fluorene	LMW (3 rings)	32.417	166
Phenanthrene	LMW	36.994	178
Anthracene	LMW	37.239	178
Fluoranthene	MMW (4 rings)	42.684	202
Pyrene	MMW	43.744	202
Benz(a)anthracene	MMW	49.461	228
Chrysene	MMW	49.624	228
Benzo(b)fluoranthene	HMW (5 rings)	54.228	252
Benzo(a)pyrene	HMW	55.627	252
Indeno(1,2,3-cd)pyrene	HMW	61.344	276
Dibenz(a,h)anthracene	HMW	61.507	278
Benzo(*ghi*)perylene	HMW	62.919	276
Acenaphthene-d10 (Surrogate)	LMW	29.567	164
Phenanthrene-d10 (Surrogate)	LMW	36.692	188
Chrysene-d12 (Surrogate)	MMW	49.354	240
Perylene-d12 (Surrogate)	HMW	55.716	264
p-Terphenyl-d14 (Internal standard)	-	43.854	244

**TMS= derivative obtained after derivatization using BSTFA. *Underlined ions were used for quantification. PAHs were classified into LMW, MMW and HMW groups for easier discussion of results*

3.1.2.4 SPE procedure and optimization

Isolute C_{18} (1000 mg, 6ml) and Isolute C_{18} (500 mg, 6ml) SPE columns were used for the extraction of the PAHs and triclosan from wastewater samples. For extraction of PAHs, a Biotage application note for analysing polyaromatic hydrocarbons (PAHs) in water was applied with some modifications (Biotage, 2016) . Briefly, isopropanol (1% v/v, added as modifier to minimize adsorption of high molecular weight PAHs onto glass surfaces due to their hydrophobicity) was added to 500 ml of filtered wastewater (influent was centrifuged at 3846 g for 15 mins at 20 ^{o}C before filtration) without pH adjustment for the extraction of PAHs in the aqueous phase, followed by addition of surrogate standards (acenaphthene-d_{10} for LMW PAHs, phenanthrene-d_{10}, for MMW PAHs, chrysene-d_{12} and perylene-d_{12} for HMW PAHs) at 200 ng/L before passing through the SPE. Cartridges (Isolute C_{18}, 1000 mg, 6 ml) were conditioned with 5 ml of isopropanol followed by 5 ml of deionized water containing 2 % isopropanol (v/v) at a flow rate of 5 ml/min. Samples were then percolated through the cartridges at a flow rate of 10 ml/min. Sample bottles were then rinsed with acetone to prevent loss of analytes to the glass walls, and then diluted with 90 ml of deionized water before passing through the cartridges - this serves as a prewash step to remove unwanted impurities including chlorophyll. The cartridge was finally washed with 10 % isopropanol (v/v) and dried under vacuum for 20 mins (cartridges were not dried longer than this to prevent loss of low molecular weight PAHs due to volatilization). Elution was performed by passing 3 ml of dichloromethane through the cartridge twice (6 ml total volume). The extract was then evaporated under a gentle stream of nitrogen at 30 ^{o}C using Labconco Rapidvap Evaporator to 1000 µl before injection into the GC-MS.

Extraction of triclosan was performed according to Kookona et al., 2011 (Kookana *et al.*, 2011) with some modifications. Briefly, methanol (1 % v/v) was added to 500 ml – 1000 ml of filtered wastewater (influent was centrifuged at 4000 rpm for 15 mins at 20 ^{o}C before filtration) and was adjusted to pH 2 by adding sulphuric acid for aqueous phase extraction. This was followed by addition of surrogate standard ($^{13}C_{12}$- Triclosan) at 100 ng/L level before passing through SPE. Cartridges (Isolute C_{18}, 500 mg, 6 ml) were conditioned with 5 ml of methanol followed by 5 ml of deionized water at a flow rate of 5 ml/min. Samples were then percolated through the cartridges at a flow rate of 10 ml/min. The cartridge was finally washed with 10 % methanol (v/v) and dried under vacuum for 30 mins. Elution was performed by passing 3 ml of ethyl acetate through the cartridge twice (6 ml total volume). The extract was then evaporated under a gentle stream of nitrogen at 30 ^{o}C using Labconco Rapidvap Evaporator to incipient dryness and re-dissolved in 900 µl of dichloromethane. The

sample was derivatized by adding BSTFA with 1% TCMS (see below for derivatization conditions) before analysing on the GC-MS.

For extraction of PAHs and triclosan in combined aqueous and particulate matter (PM) phases, 50 ml of unfiltered influent and 100 ml of unfiltered effluent was percolated through the SPE cartridge as described above.

Triclosan acidification test

In some studies, water samples were acidified to pH 2 before extraction of triclosan with SPE (Lee *et al.*, 2003; Kookana *et al.*, 2011). The importance of this pH adjustment was evaluated by comparing extraction efficiencies at pH 2 and pH 6 – 7 (the pH range of wastewater prior to modification). Triclosan was spiked at 100 ng/L into six pre-cleaned bottles filled with filtered effluent (500 ml). The first three bottles were adjusted to pH 2 with the addition of sulphuric acid, while the other three bottles were not pH adjusted. All samples were processed with SPE, and the corresponding peak areas after GC-MS analysis were used to estimate analyte recovery.

Optimization of triclosan derivatization

Derivatization of triclosan before GC analysis improves response and eradicates peak tailing (Tohidi and Cai, 2015). Therefore, BSTFA with 1% TCMS was selected as the derivatization agent for triclosan because of its strong silylation strength and versatile application (Sigma, 2011). Three derivatization methods were investigated to determine the optimum condition, and they include;

10% Derivatization concentration: 100 µl of BSTFA with 1% TCMS was added to 1ml of triclosan solution (100 ng/ml). Maintained at 60 ^{o}C for 30 mins in a test tube heating block (Sanchez-Brunete *et al.*, 2009)

50% Derivatization concentration: 50 µl of BSTFA with 1% TCMS (derivatization agent) was added to 100 µl of triclosan solution (100 ng/ml). Maintained at 60 ^{o}C for 30 mins in a test tube heating block (Zhang *et al.*, 2006)

100% Derivatization concentration: 100 µl of BSTFA with 1% TCMS was added to 100 µl of triclosan solution (100 ng/ml). Maintained at 60 ^{o}C for 30 mins in a test tube heating block.

Final extracts from the three derivation methods were injected into the GC-MS system, and the quantification ions corresponding to parent triclosan (m/z 288, 289 and 290) and

derivatized triclosan (triclosan -TMS, m/z 345, 347, 360 and 200) were monitored. Complete derivatization was established when the ions relating to parent triclosan disappeared from the spectrum as a result of their conversion to triclosan-TMS. Furthermore, derivatization of pure analytical standard was compared to that of spiked effluent to determine the conversion efficiency in a matrix sample.

3.1.2.5 Method validation study

Method accuracy was evaluated by performing recovery experiments in blanks (deionized water, n = 3) at three fortification levels (20 ng/L, 200 ng/L and 1000 ng/L) and matrix sample (final effluent) at 100 ng/L. A recovery experiment was carried out for PAH surrogates at 20 ng/L in DI water and 200 ng/L in effluent. Triclosan surrogates were spiked and recovered from DI water and effluent at 100 ng/L. The repeatability of the method was determined by the relative standard deviation (% RSD) from the recovery experiments in the fortified blank and matrix samples.

To calculate the percentage recovery of the compounds during the recovery experiment according to the US EPA 1984 (USEPA, 1995)

$$\%\, recovery = \frac{Area\ A - Area\ B}{Area\ T}\ \ x\ 100 \qquad (1)$$

Where: Area A is the area of recovered spiked sample, Area B is the area corresponding to the background concentration of the analyte in the matrix, and Area T is area corresponding to the known concentration of the spike (reference standard).

Instrumental limits of detection (IDL) and method detection limit (MDL) were determined as stated in Section 3.2.2.5. The MDL in effluent was determined using the surrogate compounds because the concentration of the analytes in effluent samples was ten times above the MDL in deionized water or five times above the instrumental detection limit, which then make its impracticable to determine MDL of the analytes in effluents (USEPA, 1995).

Standard curves and estimation of concentrations

Analytes were identified by their retention time and fragmentation ions. Multipoint calibration curves were generated by injecting reference PAH and triclosan standards in triplicate at five concentration levels: from 2 to 2000 ng/ml. Linearity was established when regression coefficient was > 0.99. To predict the concentration of an unknown analyte in a sample, an appropriate calibration model is important. Selecting an appropriate calibration model

involves a decision as to either allow the calibration curve pass through a point of intercept on the y-axis (so that y = mx + c) or force the curve to go through the origin (so that y = mx) (Dolan, 2009).

Regression statistics of the calibration data on Microsoft Excel 2013 can be used to make this decision. This decision is based on closeness of the y-intercept to zero, and can be tested statistically using standard error (SE) (Dolan, 2009). The standard error of the y-intercept (SE_y) obtained by the regression analysis is based upon the variability at the y-intercept and can be used to test if the curve passes through zero such that;

When y-intercept > SE_y, use intercept such that y = mx + c

When y-intercept ≤ SE_y, force curve through the origin, such that y = mx (c = 0).

Isotope labelled internal standards (deuterated PAHs and labelled triclosan) were selected as surrogate standards due to their similarities with the analytes of interest and absence in wastewater. These surrogates were spiked in calibration solutions and wastewater samples to correct for extraction losses and sample matrix effect.

Method 1

In the early method validation stages, quantification of triclosan in WWTP A and WWTP B was carried out using a multipoint calibration curve at five concentration levels between 0.02 – 10 µg/ml . Surrogate standard ($^{13}C_{12}$-Triclosan) was added at a known concentration (0.1 µg/ml) to the wastewater samples after extraction (i.e. just before GC injection). The peak area thus obtained is compared to that of an analytical standard of the surrogate to quantify any matrix effect. A matrix effect correction factor is established (signal suppression or enhancement), and the final concentration in the sample is calculated according to Equation 2. MFA was used as instrumental standard to monitor instrumental variations across runs.

$$Final\ concentration\ in \frac{ng}{L} = C_C\ x\ D\ x\ (100 - \%\ SS) \qquad (2)$$

Where C_c = Concentration obtained from the calibration curve, D = Dilution factor, and SS = Signal Suppression (due to matrix effect).

Method 2

This method is based on stable isotope quantification. The stable isotope quantification method implies spiking the isotope labelled standards (surrogates) into the calibration curve

solutions at a known concentration and obtaining a relative response factor (peak area of primary analyte divided by that of surrogate) to plot a multipoint calibration curve. The surrogates are also spiked into the wastewater samples before SPE extraction so that extraction efficiencies and matrix effect are accounted for. The relative response factors (RRF) obtained from the samples are used to estimate the concentration from the calibration curve, and the final concentration is calculated according to Equation 3.

$$Final\ concentration\ in \frac{ng}{L} = C_C\ x\ D \qquad (3)$$

Where C_c = Concentration obtained from the calibration curve, D = Dilution factor.

This method replaced method 1 as it effectively corrects extraction inefficiencies, and was adopted to determine the concentration of triclosan and PAHs in WWTP A.

3.1.3 **Results and discussion**

3.1.3.1 Chromatographic performance

Optimization of chromatographic separation is necessary when analysing a wide a range of PAHs (from low to high molecular weight) as this ensures good separation of compounds of interest. Employing a 60 m capillary column allowed good separation of the PAHs. The result of the GC injection liner test showed that the blue liner increased the sensitivity of the GC-MS equipment for all PAHs by 20 – 50 %, especially for the high molecular weight PAHs at low concentrations (data not shown). This helped to minimise high end discrimination associated with this type of analysis, and was subsequently routinely employed for the analysis of PAHs and triclosan throughout the study. The employed temperature program for PAHs allowed for the separation of the 15 PAHs in 62 mins, with naphthalene (Naph) eluting first at 21.0 min and benzo(ghi)perylene (BghiP) last at 61.8 min (Figure 3-1). An optimized temperature program for triclosan allowed its elution in 17.34 min. Optimum collision energy was set up at 70 eV, due to the poor fragmentation of the aromatics in EI ionization (Pitarch *et al.*, 2007).

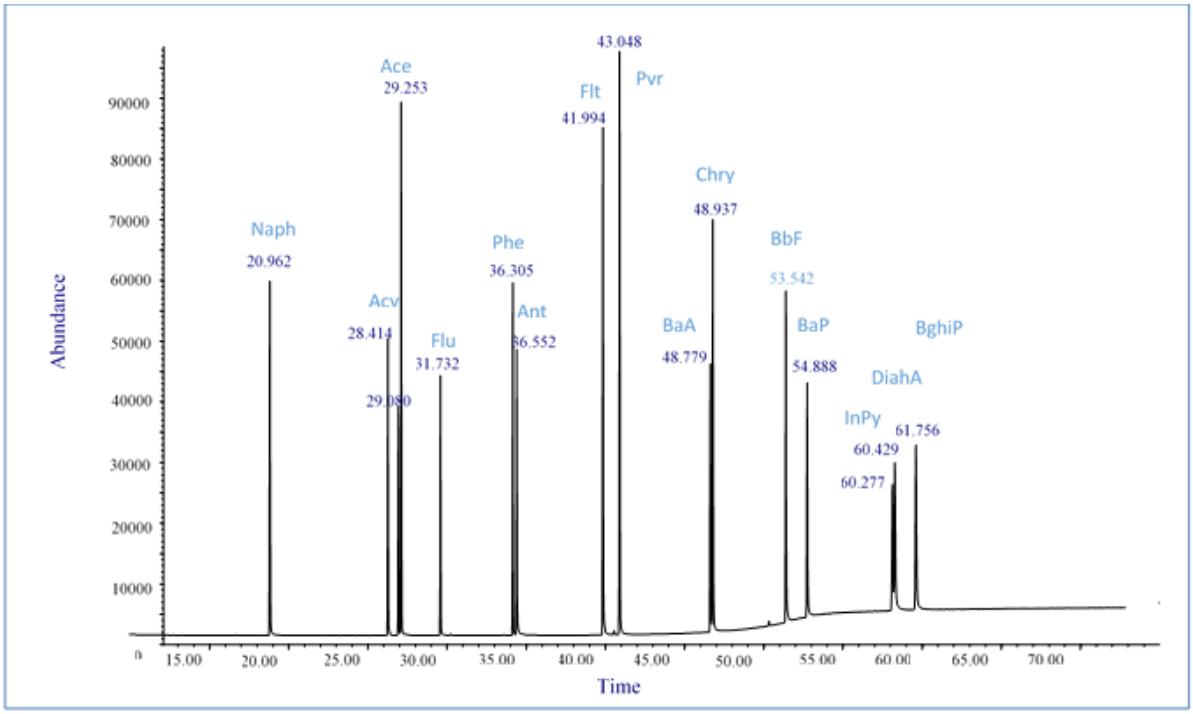


Figure 3-1 GC-MS Chromatogram of 15 priority PAHs in the analytical standard at 2000 ng/ml. The compounds are; napthalene (Naph), acenaphthylene (Acy), Acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (pyr), benz(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InPy), dibenz(a,h)anthracene (DiahA), and benzo(ghi)perylene (BghiP)

3.1.3.2 Statistical analysis of calibration curve

The result of the linear regression analysis of the calibration curve from a single run of triclosan and 15 PAHs (Table 3-3) shows that different calibration models should be used for individual analytes to achieve accurate predictions. Triclosan, Naph, Acy, BaP, InPy, DiahA and BghiP all required the curve to be forced through zero (such that y = mx) for accurate concentration estimations in an unknown sample, while the other compounds required the alternative calibration curve model (such that y = mx + c). These calibration curve evaluations were repeated several times over the course of our research and the results were consistent, even across long (18 months) time intervals (at the same multipoint calibration concentration range). Such evaluations are important. It has been reported that errors as large as 45 % can occur when quantifying the concentrations of compounds near the method limit of detection and lower limits of quantification if an incorrect calibration curve fit is applied (Dolan, 2009).

Table 3-3 Calibration curve test results, model and equations for triclosan and 15 PAHs

Compound	**R^2**	**y-intercept**	**Standard Error (SEy)**	**Calibration model**	**Calibration equation**
Triclosan	0.9989	0.152	0.191	y = mx	y = 0.0104x
Naphthalene	0.9992	0.060	0.067	y = mx	y = 0.0091x
Acenaphthylene	0.9991	0.075	0.053	y = mx + c	y = 0.0066x – 0.0746
Acenaphthene	0.9997	0.021	0.027	y = mx	y = 0.0062x
Fluorene	0.9998	0.040	0.024	y = mx + c	y = 0.0061x – 0.0398
Phenanthrene	0.9999	0.034	0.021	y = mx + c	y = 0.0067x – 0.0343
Anthracene	0.9979	0.075	0.055	y = mx + c	y = 0.0046x – 0.075
Fluoranthene	0.9981	0.094	0.068	y = mx + c	y = 0.0058x – 0.0942
Pyrene	0.9979	0.107	0.079	y = mx + c	y = 0.0064x – 0.1072
Benz(a) anthracene	0.9902	0.062	0.049	y = mx + c	y = 0.0018x – 0.062
Chrysene	0.9951	0.081	0.062	y = mx + c	y = 0.0034x – 0.0815
Benzo(b) fluoranthene	0.9983	0.039	0.029	y = mx + c	y = 0.012x – 0.0388
Benzo(a)pyrene	0.9969	0.006	0.015	y = mx	y = 0.0046x
Indeno (1,2,3-cd)pyrene	0.9951	0.003	0.006	y = mx	y = 0.0015x
Dibenz(a,h) anthracene	0.9972	0.001	0.005	y = mx	y = 0.0018x
Benzo(ghi) perylene	0.9990	0.001	0.007	y = mx	y = 0.0037x

3.1.3.3 SPE optimization and method performance

C_{18} SPE cartridges have been a popular choice in the literature for extraction of PAHs and triclosan from water samples (Ying and Kookana, 2007; Kookana *et al.*, 2011; Jones *et al.*, 2012; Qiao *et al.*, 2014). Oasis HLB is also frequently used for extraction of triclosan (Lee *et al.*, 2003; Lozano *et al.*, 2013), but is about three times more expensive than C_{18}. Therefore, the Isolute C18 cartridge was selected for the extraction of PAHs and triclosan from wastewater. The extraction procedure was optimized by testing the C_{18} cartridge for its efficiency in analyte retention. Use of Isolute C_{18} (500 mg, 6ml) resulted in poor recoveries of high molecular weight PAHs, while Isolute C_{18} (1000 mg, 6ml) performed much better. Hence, Isolute C_{18} (1000 mg, 6ml) was selected to extract PAHs and Isolute (500 mg, 6 ml) for triclosan. The US EPA recommends an analyte recovery range of 70 – 130 % with a relative standard deviation (RSD) < 20% (USEPA, 2012). Hence, extraction efficiencies were evaluated for closeness to these recommended values. The results of recovery experiments of triclosan showed that good recoveries (86 – 104%) were recorded at the three fortification levels (1000 ng/L, 200 ng/L and 20 ng/L) tested in DI water, and at 100 ng/L in effluent (102 %) (Table 3-4; See chromatogram in Figure 8-18). The C_{18} cartridge was the best choice for scientific and economic reasons as the recoveries of triclosan with the cartridge was comparable to that achieved (86 – 88% at 100 – 1000 ng/L in DI water and wastewater) by the more expensive HLB cartridges in other studies (Lee *et al.*, 2003; Lozano *et al.*, 2013). Good recoveries of 101 % and 128 % were also recorded for $^{13}C_{12}$-Triclosan in DI water and effluent respectively; hence this is an appropriate surrogate for triclosan analysis. RSDs were generally lower than 20%, thereby showing method precision.

Table 3-4 Recoveries of triclosan and surrogate at different fortification levels in deionized water (DI) and effluent, and method detection limit in DI water and effluent. Surrogate standard is indicated in italics

Compound	**Recovery in DI water (blank), % (RSD), n =3**			**Recovery in Effluent (matrix), % (RSD), n = 3**	**MDL in ng/L**	
	1000 ng/L Spike	200 ng/L Spike	20 ng/L Spike	100 ng/L Spike	Blank	Matrix
Triclosan	86 (8.8)	94 (5.1)	104 (8.8)	102 (11.8)	1.7	n/a
	100 ng/L Spike			100 ng/L Spike		
$^{13}C_{12}$-Triclosan	101 (17.2)			128 (6.3)	1.4	5.6

The 15 PAHs studied were classified according to their number of benzene rings into low molecular weight PAHs (LMW, 2 – 3 rings), middle molecular weight PAHs (MMW, 4 rings), and high molecular weight PAHs (HMW, 5 rings or more) for discussion purposes (Table 3-2). Most of the recoveries were more than satisfactory for LMW PAHs in DI water (66 – 121%) and effluent (62 – 128%) at all fortification levels (Table 3-5). At the three fortification levels (20 ng/L, 200 ng/L and 1000 ng/L) in DI water, good recoveries (101 – 133%) were recorded for all MMW PAHs except benz(a)anthracene and chrysene with recoveries of 175% and 148% respectively. However, recoveries of MMW PAHs ranged from 124 – 179% in effluent at 100 ng/L fortification level. Recovery of HMW PAHs in DI water was between 83 – 98 %, 47 – 120% and 90 – 102 % at 20 ng/L, 200 ng/L and 1000 ng/L respectively. Recovery of HMW PAHs in effluent was between 22 – 88% at 100 ng/L fortification level (see chromatogram in Figure 8-19). The recovery of some HMW PAHs (InPy, DiahA and BghiP) was not determined at 1000 ng/L in DI water as this concentration was well above their solubility- which ranged from 8.3 – 38 ng/L (Table 3-1). This poor solubility also explains the relatively poor recovery of these chemicals in effluent spiked at 100 ng/L. The recovery of the surrogate standards in effluent ranged from 76 – 123 % at 200 ng/L fortification level.

Recoveries of PAHs in effluent were corrected using the surrogate standard deuterated PAHs to achieve a better relative recovery (Table 3-5). Acenaphthene-d_{10} was used as surrogate for Naph, Acy, Ace and Flu; phenanthrene-d_{10} for Phen, Anth, Flt and Pyr; chrysene-d_{12} for BaA, Chry, BbF), and BaP; and perylene-d_{12} for InPy, DiahA and BghiP. Relative recoveries of LWM PAHs, MMW PAHs and HMW PAHs was between 82 -114 %, 110 – 160%, and 28 – 98% respectively. This approach slightly improved analyte recovery. RSDs were generally lower than 20% thereby showing method precision and reproducibility.

The MDLs were determined by a spiked concentration of 2 ng/L. As shown in Table 4, the MDLs of triclosan and $^{13}C_{12}$-Triclosan were 1.7 ng/L and 1.4 ng/L in deionized water respectively. Since MDL of triclosan could not be determined in the effluent samples due to background interference, the MDL of the surrogate ($^{13}C_{12}$-Triclosan, 5.6 ng/L) was determined. These determined MDLs passed the acceptance criteria in Section 3.2.2.5 (i.e. MDL < spike concentration or ≤ 5 times spike concentration, and ≥ spiked concentration/10). The MDL obtained was two orders of magnitude below the average annual allowable standard of 100 ng/L in freshwater as recommended by UK Technical Advisory Group on the Water Framework Directive (UKTAG, 2013).

Table 3-5 Recoveries of 15 priority PAHs and surrogate at different fortification levels in deionized water (DI) and effluent, and method detection limit in DI water and effluent. Surrogate standard is indicated in italics

Compound	**Absolute recovery in DI water (blank), Mean % (RSD), n =3**			**Recovery in Effluent (matrix), Mean % (RSD), n = 3**		**MDL in blank (ng/L)**	**MDL in matrix (ng/L)**
	1000ng/L Spike	200ng/L Spike	20ng/L Spike	100 ng/L Spike	Relative recovery*		
Naphthalene	66 (8.4)	79 (1.9)	118 (3.7)	62 (3.8)	82.4	1.2	-
Acenaphthylene	69 (11.4)	81 (2.6)	72 (1.4)	93 (5.3)	122.1	0.4	-
Acenaphthene	74 (9.0)	88 (5.3)	85 (3.5)	78 (6.5)	103.5	1.0	-
Fluorene	82 (8.7)	97 (3.7)	89 (1.7)	100 (4.4)	132.4	1.5	-
Phenanthrene	91 (8.3)	108 (4.6)	121 (0.7)	110 (4.4)	97.7	1.1	-
Anthracene	88 (9.9)	99 (3.9)	80 (1.8)	128 (6.8)	114.4	2.7	-
Fluoranthene	101 (6.8)	126 (4.3)	116 (0.6)	147 (7.6)	130.9	3.0	-
Pyrene	101 (6.7)	122 (6.1)	112 (0.6)	143 (7.1)	127.7	7.2	-
Benz(a) anthracene	122 (4.5)	175 (5.1)	133 (0.8)	179 (13.7)	160.2	3.2	-
Chrysene	102 (6.5)	148 (6.0)	109 (0.4)	124 (4.4)	109.9	3.4	-
Benzo(b) fluoranthene	100 (5.3)	137 (3.5)	96 (1.6)	88 (5.0)	106.7	7.4	-
Benzo(a)pyrene	90 (8.5)	127 (2.0)	91 (4.1)	80 (7.3)	97.8	5.9	-
Indeno (1,2,3-cd)pyrene	-+	120 (2.8)	97 (4.2)	28 (8.0)	34.1	2.7	-
Dibenz(a,h) anthracene	-+	47 (5.7)	83 (18.0)	23 (2.7)	27.9	7.4	-
Benzo(ghi) perylene	-+	78 (8.3)	98 (7.1)	23 (3.3)	28.0	2.8	-
Surrogates	**20 ng/L Spike**			**200 ng/L Spike**			
Acenaphthene-d10	65 (3.1)			76 (6.3)		0.4	0.7
Phenanthrene-d10	83 (3.6)			113 (4.3)		0.4	0.2
Chrysene-d12	184 (3.6)			123 (7.5)		3.6	10
Perylene-d12	114 (1.7)			82 (7.6)		6.7	10

***Relative recovery obtained by correcting the recoveries of the primary analytes with that of the surrogates. + Recoveries were not determined at this level as the solubility of the chemicals exceeded 1000 ng/L*

The MDLs were determined with a spike concentration of 1 – 10 ng/L from LMW – HMW PAHs in both DI water and effluent samples. The MDL of 15 priority PAHs in deionized water ranged from 0.4 – 7.4 ng/L. the MDL of isotope labelled PAHs (surrogates) ranged from 0.2 – 10 ng/L in effluent samples (Table 3-5) as high background concentrations prohibited MDL determination of the parent compounds as discussed above. These determined MDLs passed the acceptance criteria in Section 3.2.2.5. The method detection limits achieved were also below the maximum allowable concentration environmental quality standards (MAC-EQS) for naphthalene (130,000 ng/L), anthracene (100 ng/L), fluoranthene (120 ng/L), benzo(a)pyrene (27 ng/L) and benzo(b)fluoranthene (17 ng/L) as proposed in the EU Water Framework Directive (EU, 2013b). Furthermore, these MDL values are well below the reported levels of triclosan and the investigated PAHs in wastewater around the world (Kookana *et al.*, 2011; Yao *et al.*, 2012; Qi *et al.*, 2013), hence the method can be accurately used to quantify them.

Derivatization test

The derivatization test carried out for analysis of triclosan showed that 10 % derivatization was optimum to silylate all the triclosan in the sample as ions relating to parent triclosan (288, 288, and 299) completely disappeared from the spectrum. This derivatization condition was further optimized by varying the reaction time (15 mins and 30 mins). 15 minutes was observed to be sufficient and was adopted. 50 % and 100 % derivatization also gave similar results. However, 100 % derivatization showed lower peak abundance when compared to the standard - suggesting signal suppression at this condition.

Acidification test

The result of the sample acidification test (Table 3-6) showed effective triclosan recovery at pH 2 (95 % recovery) compared to the poor recovery (38 %) recorded in the non-acidified sample. This might be due to the pKa (7.9 – 8.1) of triclosan that can lead to ionization at environmental pH, thereby resulting in partial availability of triclosan in its neutral and ionized form (UKTAG, 2009). This may also have influenced the interaction of triclosan with the extraction sorbent (C_{18}) during SPE. To further explain this, protonation and micro-species distribution of triclosan at pH 1 – 14 was calculated using MarvinSketch 17. This showed that between pH 1 – 4, triclosan is 100 % in its neutral form. However, 83 % steady form triclosan (17 % in ionized form) is available at pH 7, while only 32 % is available at pH 8 (68% in ionized form). Samaras et al. also reported low recovery of triclosan at pH 7 for

C18 SPE cartridge (Samaras *et al.*, 2011). The addition of organic modifier (1% methanol v/v) to the water sample, increased extraction efficiency slightly by 3% (Table 3-6)

Table 3-6 Comparing extraction of triclosan in an acidified sample to a neutral sample, and the effect of an organic modifier. Standard deviation in bracket

	Area of standard at 100 ng/ml	**Peak area from acidified sample (pH 2) + modifier**	**Peak area from acidified sample**	**Peak area for non-acidified sample**
Triclosan	20402 (29)	19441 (153)	18353 (243)	7831 (24)

3.1.4 Application to real wastewater samples

The validated methods were employed to investigate the concentrations of triclosan and 15 priority PAHs in both the dissolved and particulate phase of the wastewater, and assess the mass removal rates achieved by the treatment plants.

3.1.4.1 Triclosan in wastewater

Table 3-7 shows the level of triclosan in the aqueous phase of the wastewater in WWTP A and WWTP B (sample collected 15th of May, 2015) as determined by quantification method 1, which involved estimation of signal suppression or enhancement and subsequent correction of final concentrations. The aqueous concentration of triclosan in WWTP A was 1313 (5.5) ng/L in influent and 256 (4.1) ng/L in the final effluent. In WWTP B triclosan concentration was lower than in WWTP A in the influent (804 ng/L) but similar to WWTP A in the final effluent (238 ng/L).

Table 3-7 Concentration of triclosan in the aqueous phase of influent and effluent of two different activated sludge WWTP in UK (WWTP A and B) - as determined by Method 1

	WWTP A			**WWTP B**		
	Influent	Effluent	% Removal	Influent	Effluent	% Removal
Triclosan concentration (ng/L)	1313 ± 5.5	256 ± 4.1	80.4 ± 1.8	804 ± 8.8	238 ± 7.8	70.2 ± 8.8
% Signal suppression	-8	49		-18	36	

Data are expressed as mean ± SD (n=3 for WWTP A, and n = 5 for WWTP B).
Reported final concentrations have been corrected for matrix effect using the surrogate ($^{13}C_{12}$- Triclosan) recovery in the matrix.

The total (aqueous and particulate matter) triclosan concentration was 13117 ng/L (13.1 µg/L) in raw influent and 1443 ng/L (1.4 µg/L) in final effluent from WWTP A (samples collected in November, 2015) (Table 3-8) using the method with stable isotope quantification. These concentrations are similar to reported levels of triclosan in unfiltered municipal wastewater around the world (Kumar *et al.*, 2010; Lozano *et al.*, 2013). The concentration of triclosan in the aqueous phase of influent (1391 ng/L) of WWTP A using this quantification method was similar to that obtained using method A (1313 ng/L) (Table 3-7) showing good agreement between both methods, although the sampling period was different. Triclosan final effluent concentrations in the aqueous phase of WWTP A (256 ng/L in May, 2015 and 142 ng/L in November, 2015) and WWTP B (238 ng/L in May, 2015) exceeded the annual mean recommended standard of 100 ng/L for triclosan in freshwater and saltwater (UKTAG, 2013), and were similar to reported levels in Canada (Lee *et al.*, 2003), Australia (Kookana *et al.*, 2011), USA (Kumar *et al.*, 2010), Germany (Bester, 2005) and UK (Sabaliunas *et al.*, 2003). The chemicals investigation program (CIP) also reported that triclosan concentration in effluent in over 50 % of the 162 England WWTPs they studied was above the proposed EQS value (Gardner *et al.*, 2012). This indicates the ineffectiveness of WWTPs around the world are ineffective at removing triclosan to environmentally safe concentrations in their current format.

About 90 % of triclosan was present in the particulate phases of influent and effluent samples (Figure 3-2). This observation is as expected due to the high Log K_{ow} value (Table 3-1) of triclosan, which results in high association with suspended solids. However, Lozano *et al*., 2013 reported that 80 % and 20 % of triclosan in influent and effluent respectively is associated to the particulate phase (Lozano *et al.*, 2013). This observed difference in the effluent partitioning might be due to the solids concentration in the sample. Triclosan removal rates from the aqueous phase by the WWTPs ranged from 70 – 89 %, and about 90 % in the total liquid phase (aqueous and particulate matter). This removal is most likely due to partitioning and settling out with sludge in the primary treatment tanks and biodegradation during secondary treatment (Lozano *et al.*, 2013). The removal rates observed in this study are similar to those achieved by other activated sludge based WWTPs in the UK as reported in literature (90 – 95 %) (Sabaliunas *et al.*, 2003; Thompson *et al.*, 2005).

Table 3-8 Concentration of triclosan and 15 priority PAHs in influent and effluent of a UK activated sludge WWTP (WWTP A) - as determined by Method 2

Compound	Influent		Effluent		% Aqueous phase Removal	% total Removal
	Total (ng/L)	Aqueous phase (ng/L)	Total (ng/L)	Aqueous phase (ng/L)		
Triclosan	13117 ± 1030	1391 ± 58	1443 ± 43	142 ± 6.2	89.0	88.9
Naphthalene	689.4 (44.5)	108.6 (20.4)	211.1 (25.2)	16.3 (0.5)	85.0	69.5
Acenaphthylene	191.5 (15.7)	39.6 (2.5)	63.3 (3.0)	12.3 (0.2)	68.9	66.9
Acenaphthene	182.9 (24.9)	45.0 (5.0)	35.1 (5.0)	4.7 (0.1)	89.6	81.0
Fluorene	277.8 (33.0)	48.0 (3.4)	47.8 (1.4)	8.4 (0.3)	82.4	82.8
Phenanthrene	633.7 (61.1)	77.7 (0.7)	87.7 (21.3)	10.2 (0.1)	86.9	86.2
Anthracene	421.1 (18.2)	47.4 (1.4)	95.3 (8.3)	19.2 (1.6)	59.4	77.3
Fluoranthene	743.1 (46.5)	75.0 (2.8)	101.4 (2.0)	18.6 (0.2)	75.2	86.4
Pyrene	691.2 (21.9)	68.3 (1.5)	99.8 (1.0)	18.2 (0.1)	73.3	85.6
Benz(a) anthracene	754.4 (47.6)	111.1 (1.2)	181.3 (0.4)	36.2 (0.1)	67.4	76.0
Chrysene	556.2 (58.5)	68.2 (1.8)	129.5 (0.6)	25.5 (0.7)	62.6	76.7
Benzo(b) fluoranthene	238.6 (58.5)	15.7 (3.1)	65.4 (11.1)	6.3 (1.1)	59.8	72.6
Benzo(a)pyrene	355.3 (75.7)	12.9 (3.4)	266.3 (31.2)	4.2 (1.1)	67.5	22.9
Indeno (1,2,3-cd)pyrene	-	91.4 (4.9)	-	11.6 (4.6)	87.3	-
Dibenz(a,h) anthracene	590.1 (179)	39.1 (9.1)	273.5 (24.5)	7.7 (2.1)	80.3	53.7
Benzo(ghi) perylene	241.8 (44.1)	11.3 (0.4)	210.2 (35.4)	10.0 (3.0)	11.5	13.2
∑LMW PAHs	2396.3	366.2	539.7	71.1	80.6	77.5
∑MMW PAHs	2744.3	322.5	511.3	98.6	69.4	81.4
∑HMW PAHs	1425.9	170.4	822.7	39.8	76.7	42.3
∑PAHs	6566.5	859.1	1873.7	209.4	75.6	71.5

3.1.4.2 PAHs in wastewater

The overall concentrations of PAHs and their individual abundances in wastewater from WWTP A are shown in Table 8. All 15 PAHs investigated were detected in both influent and effluent samples. The average total (aqueous and particulate phase) concentration of PAHs in influent was (6566.5 ng/L), with middle molecular weight MMW PAHs (2744.3 ng/L), low molecular weight (LMW) PAHs (2397.3 ng/L) and high molecular weight (HMW) PAHs (1425.9 ng/L) accounting for 42 %, 36 % and 22 % respectively (Table 3-8). The total concentration of PAHs in final effluent was 1873.7 ng/L, with HMW PAHs, LMW PAHs and MMW PAHs representing 44 %, 29 % and 27 % respectively. MMW PAHs have been reported to be the most abundant PAHs in raw wastewater, while LMW PAHs were the most abundant in the effluent (Busetti *et al.*, 2006).This distribution mostly depends on the source of the PAHs entering the WWTP. The total (aqueous and particulate phase) concentrations of

PAHs in influent and effluent observed in the present study are similar to reported levels in China (Qi *et al.*, 2013) and Greece (Manoli and Samara, 2008), but 10 folds higher than those reported in Italy (Busetti *et al.*, 2006; Fatone *et al.*, 2011) and lower than levels reported in Spain (Sánchez-Avila *et al.*, 2009). The mean concentration of PAHs in the aqueous phase (Table 3-8, Figure 3-3) of the influent (851.1 ng/L) and effluent (209.4 ng/l) samples was also similar to influent levels generally reported around the world (Cao *et al.*, 2005; Yao *et al.*, 2012; Qi *et al.*, 2013), but five to ten times lower than levels reported by Wang et al., 2013 in China (Wang *et al.*, 2013c). Naph, BaA and InPy were the most abundant LWM, MMW, and HMW PAH respectively.

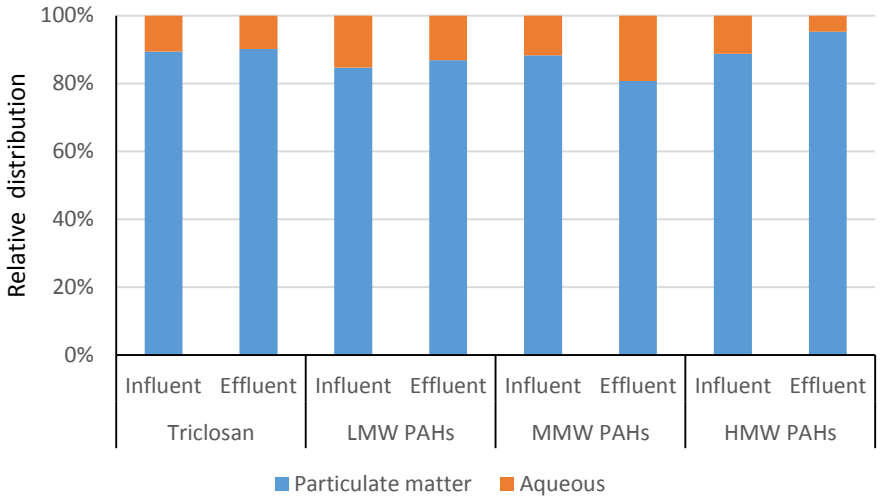


Figure 3-2 Partitioning of triclosan, LMW PAHs, MMW PAHs and HMW PAHs in the particulate and dissolved phases of influent and effluent samples from a UK WWTP (WWTP A)

The total effluent concentrations of those PAHs falling under the regulation of the EU WFD (Naph, Anth, Flt, BbF, BaP and BghiP; Table 8) were below the mean allowable concentration EQS for inland surface waters for four PAHs, Naph, Anth, Flt and BaP (MAC-EQS values of 130, 000 ng/L, 100 ng/L, 120 ng/L, and 270 ng/L respectively; (EU, 2013a), but were four to thirty times higher for two PAHs, BbF and BghiP (17 ng/L and 8.2 ng/L respectively).Therefore, this effluent poses risk to aquatic organisms when discharged in the receiving river. The concentration of BbF and BghiP in over 50 % of the 162 WWTPs monitored in England was higher than their proposed EQS standards as reported by the CIP (Gardner *et al.*, 2012)

About 83%, 88% and 88% of LMW PAHs, MMW PAHs, and HMW PAHs respectively were present in the particulate phase of the influent sample, while 86% of LMW PAHs, 80% of MMW PAHs and 95% of HMW PAHs was partitioned onto the particulate phase of the effluent sample (Figure 3-2). This is as expected, due to the high Log K_{ow} values of the PAHs (Table 3-1). The higher the Log K_{ow} of the PAH group, the more they partition onto the particulate matter present in the wastewater (i.e. HMW PAHs partitioned more to the particulate matter than LMW PAHs) (Figure 3-2). The WWTP achieved a total PAH removal rate of 72% and aqueous phase removal of 75%; this removal is most likely mainly by sorption onto solids in the sedimentation tanks, with volatilization and biodegradation contributing less (Lei *et al.*, 2007; Liu *et al.*, 2011). Only about 1 – 2 % of PAHs is reported to be removed by conventional wastewater treatment plants via volatilization (Manoli and Samara, 2008).

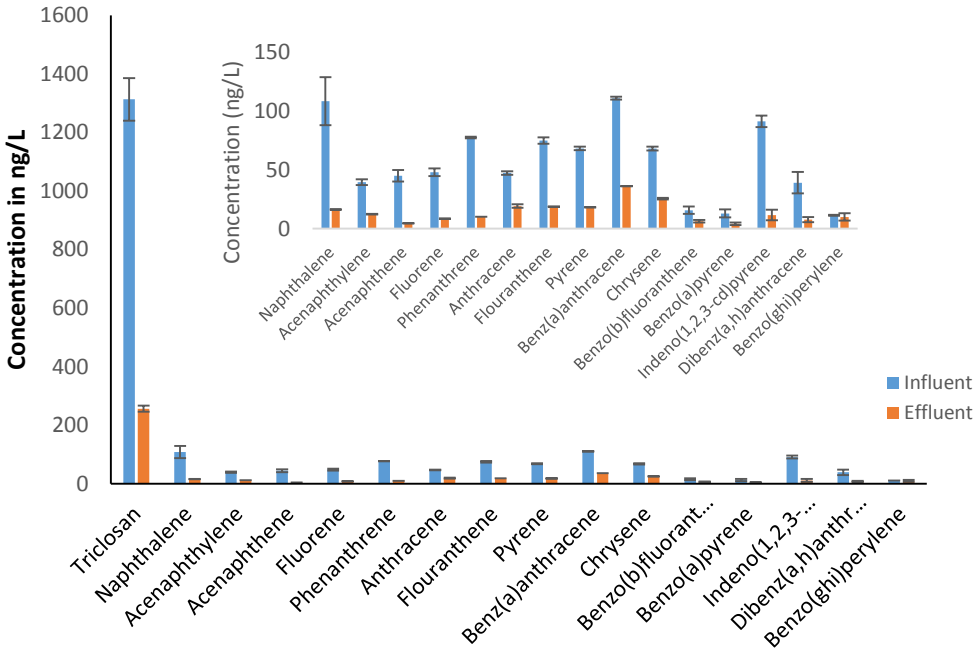


Figure 3-3 Concentration and distribution of triclosan and 15 priority PAHs in the aqueous phase of influent and effluent samples from WWTP A

3.1.5 **Conclusion**

In this study, two analytical methods based on solid phase extraction (for analyte extraction and sample concentration) and GC-MS analysis (for analyte identification and quantification) were validated for the analysis of triclosan and 15 priority PAHs in wastewater. The extraction procedure was optimized by evaluating optimum sample pH (for triclosan) and a comparison of SPE cartridge sorbents for PAHs. Extraction of triclosan was more effective at pH 2 than at pH 7, while PAH recovery in wastewater was optimal using a SPE C_{18} cartridge with a 1000 mg sorbent capacity and 500 ml of sample. Furthermore, chromatographic performance was improved by optimizing triclosan derivatization conditions, and reducing high end discrimination of high molecular weight PAHs by selecting an appropriate injection liner. The low detection limits achieved allowed for the determination of triclosan and PAHs at environmentally relevant levels, and, importantly, were below the proposed MAC-EQSs for PAHs set by the EU Water Framework Directive and below the recommended allowable concentration for triclosan advised by UK TAG. Hence, this method can be used to evaluate compliance to these standards.

The methods were employed for the quantification of triclosan and PAHs in the influent and effluent of UK activated sludge based municipal wastewater treatment plant. Triclosan and 15 PAHs were detected in the aqueous and particulate matter of both influent and effluent samples. The total concentration of triclosan in effluent of WWTP B was about tenfold higher than the UK TAG allowable concentration, despite the 89% removal achieved by the plant. HMW PAHs were the most abundant in effluent from this WWTP followed by LMW PAHs and MMW PAHs, with concentrations (total) of BaP, BbF and BghiP particularly higher than their MAC-EQSs. However, in the aqueous phase of the effluent, HMW PAHs were the least abundant suggesting they are mostly bound to the particulate matter. Removal of triclosan and PAHs in this WWTP was most likely due to sorption onto sludge during primary and secondary treatment. Despite the ineffective removal achieved by this WWTP, this issue is worse in urban areas with inadequate wastewater treatment as they might not be afforded the advantage of reduced environmental pollution by these chemicals.

3.2 Quantification of polybrominated diphenyl ether (PBDE) congeners in wastewater by gas chromatography with electron capture detector (GC-ECD)

3.2.1 Introduction

Brominated flame retardants (BFRs) are added to many household items such as furniture, upholstery, plastic, electronic devices and textiles; they consist of several groups of compounds, including polybrominated diphenyl ethers (PBDEs), hexabromoclyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA) (Rahman *et al.*, 2001; Xie *et al.*, 2007; USEPA, 2017). PBDEs are the second most produced BFR in the world, accounting for 33% of global production, while TBBPA accounts for 60% (Labadie *et al.*, 2010). According to the United Nations Environment Programme, the total global production of all PBDEs from 1970 to 2005 was between 1.3 million and 1.5 million tonnes with deca-mix BDE formulation accounting for about 85 % of this number (UNEP, 2017). PBDEs are persistent and hydrophobic compounds that tend to bio-accumulate; their occurrence in the environment has been of growing concern due to their toxicological effects including disruption of the thyroid hormone function in humans and wildlife, which has led to a ban on their production and usage in the EU and the USA (Labadie *et al.*, 2010; Gorga *et al.*, 2013). In the EU, production, import and use of commercial PBDE formulations, including pentaBDE and OctaBDE, was banned in 2003 (Cristale *et al.*, 2012; Hutzinger, 2013), and this was extended to a worldwide ban in 2009 at the Stockholm Convention of Persistent Organic Pollutants (UNEP, 2017). Despite this ban, these PBDEs are still being deposited into the environment, as they are not covalently bound to the applied products and are released with usage (Siddiqi *et al.*, 2003; Kim *et al.*, 2013). Deca-BDE formulation is currently being phased out in the EU and US, but still extensively produced in China and the resulting products are distributed globally (Xiang *et al.*, 2014).

Effluents from WWTPs have been identified as a major source of PBDE into the environment. WWTPs receive PBDEs through municipal wastewater (discharged during production, application and release from in-use domestic products) and surface runoff. (Kim *et al.*, 2013; Xiang *et al.*, 2014). Therefore, an effective analytical methodology to extract and quantify the presence of PDBEs in wastewater is required.

Several methods, including conventional liquid-liquid extraction (LLE), pressurized liquid extraction (PLE), solid phase extraction (SPE), solid phase micro-extraction (SPME) ultrasound assisted extraction and cloud point extraction (CPE) have been used to extract PBDEs from water and semi-solids (Fontana *et al.*, 2009; Covaci *et al.*, 2010; Labadie *et al.*,

2010). Most of these methods have limitations, including excessive use of solvents, and/or require further sample clean up using multilayer column chromatography or gel permeation chromatography to reach lower detection limits (Covaci *et al.*, 2010; Daso *et al.*, 2012). SPE is the most common extraction method used today as it offers low solvent usage and broad applicability. Careful selection of appropriate sorbent and an optimized elution protocol can produce clean extracts that require no further clean up (Sánchez-Avila *et al.*, 2009; Cristale *et al.*, 2013). For unequivocal identification and quantification of brominated flame-retardants including PBDEs in environmental samples, gas chromatography coupled with mass spectrophotometry (GC-MS) operating in either electron ionization (EI) or electron capture negative ionization (ECNI) mode is often used (Mai *et al.*, 2005; Fontana *et al.*, 2009; Lee *et al.*, 2014). However, GC-ECD (electron capture detector) has also been employed for quantification of PBDEs in environmental samples (De Wit, 2002; Li *et al.*, 2009; Daso *et al.*, 2012).

Determination of PBDEs in environmental samples is most commonly carried out by GC-MS mainly because of its selectivity, but GC-ECD is advantageous as it is cheaper, more user friendly and more sensitive, due to lower detection limits (Stapleton, 2006). However, the ECD is prone to halogenated interference, since identification and resulting quantification of compounds is solely based on retention time; hence ECD based methods suffer from limited selectivity (Stapleton, 2006). This shortcoming of the GC-ECD method can, however, be effectively minimised by carefully selecting GC columns and a clean-up method that produces high quality extracts free of interfering compounds. GC-ECD system is cheaper than GC-MS systems for micropollutant analysis in terms of purchasing, maintenance and costs per sample. A basic model GC-ECD system by Agilent Technologies costs half the price of a GC-MS system in both capital (about $45,000 for GC-ECD and $91,000 for GC-MS) and maintenance cost ($1200 for GC-ECD and $2600 for GC-MS per maintenance visit). Additionally, private laboratories carrying out analysis of total polychlorinated biphenyls (PCB) and congeners in soil and sediments reported that per sample GC-MS costs ($500 - $1000) are twice those of GC-ECD ($250 - $750) (Price *et al.*, 2000).

Only a few researchers have applied GC-ECD to analyse PBDEs in water and wastewater (Li *et al.*, 2009; Daso *et al.*, 2012), and none of them has used SPE for sample concentration and extraction. The authors from previous studies employed LLE, CPE or molecularly imprinted SPME and were unable to analyse BDE 209 (which is a major component of the decaBDE mix mentioned above) together with other lower molecular weight congeners in one single run: a separate GC capillary column and temperature program was generally required.

Although, analysing BDE 28 to BDE 209 in a single run has been reported using GC-MS based method; such feat has not been achieved with GC-ECD (Peng *et al.*, 2009; Sánchez-Avila *et al.*, 2009). In this work, a cost-effective yet functional analytical technique using the combination of SPE and GC-ECD for the determination of selected low to high molecular weight PBDE congeners (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, and BDE 209) in wastewater was developed; special attention was given to the determination of BDE-28 to BDE 209 in a single run. The method, which involved using a multilayer SPE column for extraction and analytical performance, was evaluated on the basis of its detection limits, linear working range and repeatability. The method was then applied to determine the levels and removal of PBDEs in a municipal wastewater treatment plant in Northern England.

Table 3-9 Names, abbreviations, physical and chemical properties of PBDE congeners investigated in this study.

PBDE	Acronym	Molecular formula	Molecular weight (g/mol) [b]	Solubility (g/L) at 25ºC, pH 7 [b]	Log *K*ow [a]
2,4,4'-TriBDE	BDE 28	$C_{12}H_7Br_3O$	406.90	7.7×10^{-4}	5.88
2,2',4,4'-TetraBDE	BDE 47	$C_{12}H_6Br_4O$	485.79	2.5×10^{-4}	6.77
2,2',4,4',5-PentaBDE	BDE 99	$C_{12}H_5Br_5O$	564.69	6.2×10^{-5}	7.66
2,2',4,4',6-PentaBDE	BDE 100	$C_{12}H_5Br_5O$	564.69	7.3×10^{-5}	7.66
2,2',4,4',5,5'-HexaBDE	BDE 153	$C_{12}H_4Br_6O$	643.58	1.6×10^{-5}	8.55
2,2',4,4',5,6'-HexaBDE	BDE 154	$C_{12}H_4Br_6O$	643.58	1.9×10^{-5}	8.55
2,2',3,4,4',5',6-HeptaBDE	BDE 183	$C_{12}H_3Br_7O$	722.48	5.6×10^{-6}	9.44
DecaBDE	BDE 209	$C_{12}Br_{10}O$	959.17	1.4×10^{-6}	12.11

[a] Adopted from (Sánchez-Avila *et al.*, 2009), [b] (Cristale *et al.*, 2012)

3.2.2 **Experimental**

3.2.2.1 Materials and reagents

A certified standard solution mix of PBDEs (> 98% purity) containing eight primary congeners including BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183 and BDE 209 was obtained from Accustandard Inc via Kinesis (UK). The concentration of the congeners was 2.5 µg/ml in isooctane, except BDE 209 which was present at 25 µg/ml. BDE 77 (50 µg/ml in isooctane), PCB 209 (10 µg/ml in heptane) and 4PC-BDE-208 (50 µg/ml in toluene), which were used as surrogate standards, were purchased from Accustandard (via Kinesis UK), Sigma Aldrich (UK), and Wellington Laboratories (via Greyhound Chromatography UK) respectively with purities higher than 98%.

Stock solutions were prepared by dissolving the reference and surrogate standard in acetone (at 500 ng/ml). Working solutions were then prepared by diluting the stock solutions in acetone for sample fortification and in ethyl acetate for instrumental analysis. All solutions were stored at 4 ^{o}C, and were allowed to reach room temperature for 15 minutes before use. Ultra-trace grade of acetone, ethyl acetate and isopropanol were obtained from Sigma Aldrich (UK). Oasis HLB cartridges (200mg, 6cc) were purchased from Waters (UK); Isolute C_{18} (1g, 6 ml), ABN and Isolute PAH cartridges (1.5g, 6 ml) were from Biotage (UK).

3.2.2.2 Sample collection

Wastewater samples (raw influent and final effluent) were collected from a nitrifying activated sludge treatment plant in North East England with a population equivalent of 22,500. Samples were collected in cleaned and disinfected (with 1% Virkron for 24 hours, then rinsed multiple times with distilled water to get rid of chlorine residues) HDPE containers - analysis of containers did not show any contamination with target compounds. Samples were stored at 4 ^{o}C upon arrival to the laboratory and were used within 24 hours. To account for the concentration of the chemicals in both aqueous and particulate matter, half of the samples were filtered through glass microfiber filter to estimate the concentration of the chemicals in the aqueous phase alone (Sartorius MGB filters, 0.7mm thick, 1.0 µm particle retention) before being passed through the SPE cartridges. The other half of the samples was not filtered before SPE.

3.2.2.3 GC Instrumentation

Analyses were performed on an Agilent 7890A gas chromatography system equipped with micro electron capture detector (μECD) and an Agilent 7683B automatic injector. A DB-5MS fused silica capillary column (15m x 0.25mm I.D. x 0.1μm film thickness) from J & W Scientific, USA was used for chromatographic separation. The inlet was fitted with an SGE single taper deactivated glass liner and samples were injected (1 μl) in split/splitless mode (1 min. splitless, then 30ml/min split). The inlet and detector temperature was set at 300 ºC and 290 °C respectively. H_2 (99.999%) was used as carrier gas in constant flow mode (flow-rate 1ml/min, velocity 91.3 cm sec^{-1}). The μECD was used with N_2 (99.999%) make-up gas at a flow-rate of 30 ml/min. The GC oven was heated from 100 °C (initial hold time 1 min) to 150 °C (hold time 0 min) at 50 °C/min. and then to 290 °C (final hold time 15 min) at 12.5C/min. Data was acquired and processed using a Thermo Atlas chromatographic data system (version. 8.3). Analytes were identified solely by their retention time and quantified by their integrated peak area.

The retention time of all eight PBDE congeners was confirmed using an Agilent 7890A GC split/split less injector linked to an Agilent 5975C MSD to ensure correct identification of analyte. This GC-MS was equipped with the same DB-5MS capillary column (15m x 0.25mm I.D. x 0.1μm film thickness) used above. Analysis was done in EI mode, full scan and SIM spectrum was acquired. Ions (m/z) corresponding to the fragmentation of each BDE congeners was monitored to add a level of confidence in the identification of the analytes (Table 3-10).

Table 3-10 Comparison between the retention times and monitoring ions of PBDE congeners of interest using GC-MS and GC-ECD.

Analyte	GC-ECD t_R (min)	GC-MS t_R (min)	EI-MS/SIM (m/z)	
			Ion 1	Ion 2
BDE 28	5.26	5.35	246	246
BDE 47	6.69	6.83	326	324
BDE 99	7.76	7.92	405.5	403.5
BDE 100	8.13	8.30	405.5	403.5
BDE 153	8.98	9.17	483.5	481.5
BDE 154	9.47	9.66	483.5	481.5
BDE 183	10.67	10.87	563.5	561.5
BDE 209	16.08	16.76	811.5	809.5

t_R = retention time

3.2.2.4 SPE procedure and optimization

To achieve the low detection limits required for the quantification of analytes at environmental levels, the performance of SPE cartridges including Oasis HLB (200mg, 6ml), Isolute C18 (1g, 6ml) and Isolute PAH (1.5g, 6ml) was evaluated for the extraction of PBDEs from wastewater samples. It was not practical to match the sorbent quantity for the cartridge types, as these were the maximum sorbent amount packed into the different cartridges by the manufacturers. The extraction procedure was modified from a Biotage application note for analysing polycyclic aromatic hydrocarbons (PAHs) in water (Biotage, 2016). Briefly, isopropanol (1% v/v) was added to 100 ml of filtered wastewater sample without pH adjustment for extraction of PBDEs in the aqueous phase, followed by addition of surrogate standards at 5 ng/L each for BDE 77, PCB 209 and 50 ng/L for 4PC-BDE-208, before passing through the SPE. Cartridges were conditioned with 5 ml of isopropanol followed by deionized water containing 2 % isopropanol (v/v) at a flow rate of 5 ml/min. Samples were then passed through the cartridges at a flow rate of 10 ml/min.

Sample bottles were then rinsed with acetone to prevent loss of analyte to the glass walls, and then diluted with 90 ml of deionized water before passing through the cartridges - this serves as a prewash step to remove unwanted impurities including chlorophyll. The cartridge was finally washed with 10 % isopropanol (v/v) and dried under vacuum for 45 minutes. Elution was performed by passing 5 ml of ethyl acetate through the cartridge twice (10 ml total volume). The extract was then evaporated under a gentle stream of nitrogen at 30 ^{o}C using Labconco Rapidvap Evaporator to 500 µl before injection into the GC-ECD. Furthermore, between 50 ml – 200 ml of spiked water samples were tested, and 100 ml sample load performed better in terms of adequate analyte extraction/retention and low background noise. Different volumes of ethyl acetate were also tested to optimize analyte elution, and 10 ml (5 ml twice) showed optimum result and was employed in further work. For extraction of PBDEs in both the aqueous and particulate matter (PM) phase, 20 ml and 50 ml of unfiltered influent and effluent was diluted to 100 ml with deionized water respectively, and was processed as the filtered samples above.

3.2.2.5 Method validation study

Analytes were identified mainly by their retention time and the calibration curves were generated by injecting reference PBDE standard in triplicate at five concentration levels: from 0.5 to 10 ng/ml for all PBDEs except BDE 209 which ranged from 5 – 100 ng/ml, because of its lower sensitivity to the ECD detector and relatively higher environmental concentrations. Linearity was observed when the regression coefficient was > 0.99. PCBs have similar physical-chemical properties to BDEs (Tittlemier *et al.*, 2002), therefore rare PCB congeners can be used as surrogates for PBDE determination. BDE 77 and PCB 209 were primarily selected for internal standard quantification for BDE 28 to BDE 183 because of their absence from wastewater; these compounds have been used for this purpose in previous studies (Sánchez-Avila *et al.*, 2009; Daso *et al.*, 2012; Deng *et al.*, 2015). However, BDE 77 was dismissed as a surrogate when initial analysis of environmental samples showed its presence in wastewater. 4PC-BDE-208 was employed as surrogate standard for BDE 209. This compound was proposed by Wellington Laboratories (Canada) as the perfect surrogate for decaBDEs when an instrumental method without the capability to differentiate between mass-labelled and parent compound is employed, such as a GC-ECD method (Laboratories, 2009). Additionally, the similarity in structure/chemical composition to BDEs, and absence in environmental samples supports its suitability as a surrogate standard. The effect of complex sample matrix on GC-ECD analysis was investigated by spiking the PBDE congeners at 10 ng/L into deionized water (DI), influent and effluent (n = 3). The spiking was performed after sample extraction with SPE and prior to GC sample injection. DI water was used as blank/absolute recovery and this recovery was compared to recoveries recorded in the influent and effluent samples.

Method accuracy was evaluated by performing recovery experiments in blanks (deionized water, n = 3) and matrix samples (final effluent, n = 3) at two fortification levels (3 ng/L and 10 ng/L, 30 ng/L and 100 ng/L for BDE 209). The repeatability of the method was determined by the relative standard deviation (% RSD) from the recovery experiments in the fortified blank and matrix sample (Nácher-Mestre *et al.*, 2010; USEPA, 2012). Furthermore, statistical analysis of the calibration curve models were performed as described in Section 3.1.2.5.

Instrumental limits of detection (IDL) were established as the lowest analyte concentration that gave a signal to noise ratio of three (s/n = 3) upon the injection of standard solutions, and was determined on the Atlas software. The method detection limit (MDL) was determined according to EPA method 1984 (USEPA, 1995). Briefly, analytes were spiked at a concentration of between one to five times of IDL in blank (n = 7) and matrix sample (n = 7), then analysed on the GC-ECD. The resultant standard deviation was multiplied by the students' T-value that corresponds to six degrees of freedom to estimate the MDL.

$$MDL = T_{(n-1,1-\propto=0.99)} * (S)$$

Where: $T_{(n-1,1-\propto=0.99)}$ = students' T value for a 99% confidence level, and a standard deviation estimate with n – 1 degrees of freedom.

S = standard deviation of replicate analyses.

The acceptance criteria is that the MDL should be < spike concentration or ≤ 5 times spike concentration, and ≥ spiked concentration/10 to be accepted.

The method quantification limit (MQL) was set at three times the MDL.

3.2.3 **Results and Discussion**

3.2.3.1 Chromatographic performance

Split-less injection mode is preferred for PBDE analysis due to the relatively low environmental levels of these compounds, and a high injection temperature (300 ºC and above) is recommended to minimise discrimination of high molecular weight congeners and thermal degradation (Stapleton, 2006). Chromatographic separation optimization is necessary when analysing a wide range of BDE congeners (from triBDEs to decaBDEs) as this ensures good separation of compounds of interest when using a one-column approach. Employing a short column (15 m) with a short internal diameter (≤ 0.25 mm) allowed the detection and quantification all eight BDE congeners including BDE 209 that easily degrades in GC column when subjected to high temperatures, without compromising separation, as reported by other authors (Covaci *et al.*, 2010; Hutzinger *et al.*, 2013). Using a relatively high carrier gas flow rate further helped to reduce the degradation of BDE 209 within the GC inlet and column. This optimization made it possible to use one GC column instead of two separate GC columns: one for lighter BDEs and one for BDE 209, as used in previous studies for GC-ECD analysis (Li *et al.*, 2009; Daso *et al.*, 2012). The optimized temperature program in addition to the capillary column applied allowed for the separation of the BDE congeners in under 16.5 min, with BDE 28 eluting first at 5.3 min and BDE 209 at 16.4 mins (Figure 3-4; Figure 3-5).

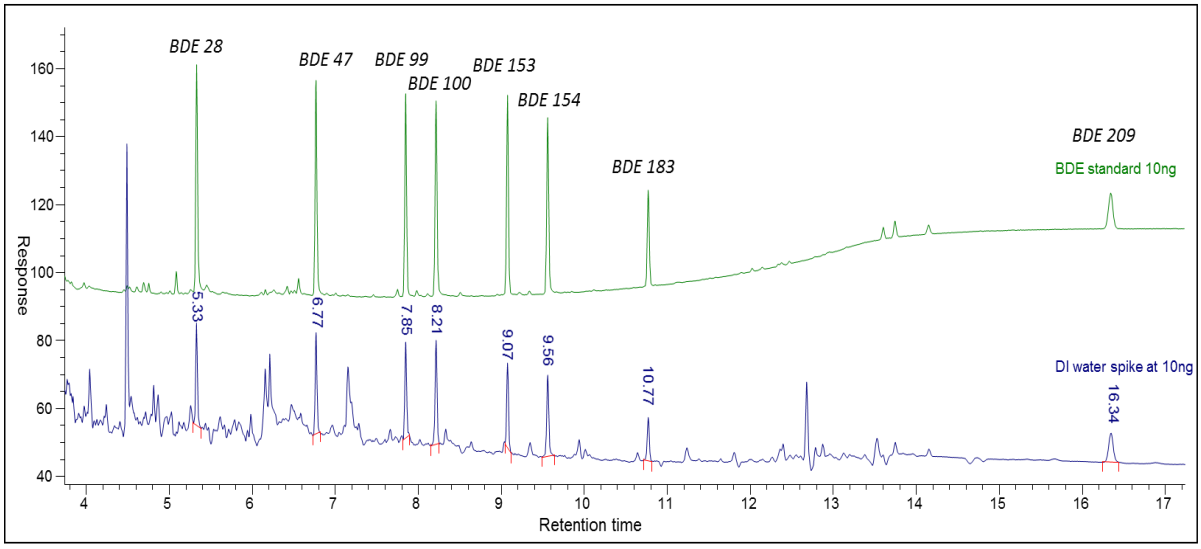


Figure 3-4 GC-ECD Chromatogram of eight primary BDE congeners in deionized water (blue trace) spiked at 10 ng/L (except BDE 209 spiked at 100 ng/L) over-layed by PBDE analytical standard (green trace). *The PBDEs were spiked before SPE.*

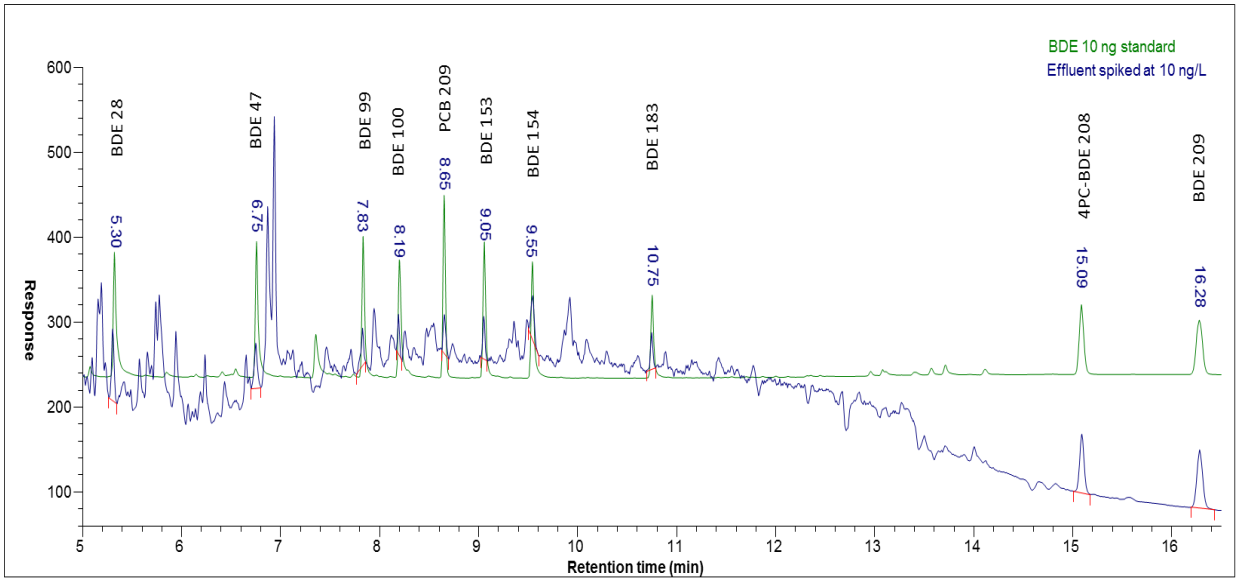


Figure 3-5 GC-ECD Chromatogram of eight primary BDE congeners and two surrogate standards in effluent (blue trace) spiked at 10 ng/L (except BDE 209 and 4PC-BDE-209 spiked at 100 ng/L) over-layed by PBDE analytical standard (green trace). The PBDEs were spiked before SPE.

3.2.3.2 Statistical analysis of calibration curve

Appropriate calibration curve equations (Table 3-11) were used to predict the concentrations of individual PBDE congeners to ensure accurate concentration predictions especially at low concentrations as described in Section 3.1.2.5. The calibration models for PBDEs were consistent across runs for over 12 months.

Table 3-11 Calibration curve test results, and equations for the 8 PBDE congeners

Compound	R^2	y-intercept	Standard Error (SEy)	Calibration model	Calibration equation
BDE 28	0.9992	0.106	0.049	y = mx + c	y = 0.1335x – 0.1065
BDE 47	0.9991	0.150	0.078	y = mx + c	y = 0.1391x – 0.1497
BDE 99	0.9980	0.150	0.083	y = mx + c	y = 0.1391x – 0.1501
BDE 100	0.9982	0.157	0.070	y = mx + c	y = 0.1242x – 0.1568
BDE 153	0.9993	0.010	0.045	y = mx + c	y = 0.0061x – 0.0398
BDE 154	0.9988	0.122	0.053	y = mx + c	y = 0.1152x – 0.1220
BDE 183	0.9978	0.085	0.042	y = mx + c	y = 0.0670x – 0.0847
BDE 209	0.9995	0.045	0.046	y = mx + c	y = 0.0058x – 0.0942

3.2.3.3 SPE optimization and method performance

The extraction procedure was optimized by testing different SPE cartridges for their efficiency in analyte retention. Cartridges tested include Oasis HLB (200 mg), HLB prime, C18 (1 g), and Isolute PAH (1.5 g). Oasis HLB and C18 have been popular choices in the literature for extraction of BDEs from water samples (Sánchez-Avila *et al.*, 2009; Cristale *et al.*, 2013; Deng *et al.*, 2015). The result of preliminary recovery experiments of eight PBDE congeners spiked in water samples showed that Isolute PAH performed better, especially in filtered effluent samples (see chromatogram in Figure 3-5), as recovery of analytes was between 69 – 126 % with a relative standard deviation (RSD) of less than 13% (Table 3-12). The mean recoveries in effluent from the Isolute PAH were significantly greater in effluent than those from the Oasis HLB (Mann-Whitney test, $P < 0.05$) and greater than Isolute C18, though only slightly insignificantly so (Mann-Whitney Test, $P = 0.06$). The recoveries were not significantly different in DI water. Extracts from the Isolute PAH cartridge showed the least interference and lower background noise. The cartridge comprises of an octadecyl layered (1000 mg) with an amino based sorbent (500 mg) that helps remove polar interferences such as humic acids from the effluent according to Biotage (the cartridge manufacturer), thereby reducing background noise and improving analyte recovery. Therefore, the Isolute PAH was selected for further optimization and subsequent analysis.

The matrix effect test showed that peak signals of the PBDE congeners were enhanced in influent and effluent samples, except BDE 99, which was suppressed in effluent (Figure 3-6). The matrix effect was about -31% (BDE 99) to 91% (BDE 209), and 3.2% (BDE 99) to 65% (BDE 209) in influent and effluent samples respectively. BDE 209 was affected the most by the matrix effect as it elutes at the end of the spectrum (Figure 3-5). The matrix effect also appears to be generally higher in influent than effluent, which might be expected due to the presence of more organic matter. This observed signal suppression or enhancement further echoes the necessity of surrogate standards for accurate quantification of PBDE congeners in wastewater.

Table 3-12 Recoveries and relative standard deviation (RSD) of PBDEs spiked in deionised water and effluent at 10 ng/L. RSD in bracket

BDE	Recoveries in DI water (%)			Recoveries in effluent (%)		
	Isolute C18	Isolute PAH	Oasis HLB	Isolute C18	Isolute PAH	Oasis HLB
BDE 28	85 (1.7)	54 (0.7)	60 (7.1)	71 (12.5)	78 (5.8)	46 (8.1)
BDE 47	75 (8.2)	61 (13.9)	65 (7.5)	84 (10.9)	79 (0.6)	44 (13.1)
BDE 99	76 (16.7)	73 (1.6)	70 (9.1)	63 (2.1)	82 (0.8)	52 (10.6)
BDE 100	71 (17.8)	58 (3.3)	79 (4.5)	52 (1.0)	69 (0.2)	33 (0.6)
BDE 153	59 (18.1)	56 (0.9)	53 (10.1)	45 (2.5)	94 (8.9)	42 (1.8)
BDE 154	58 (19.6)	53 (1.2)	51 (6.3)	70 (27.2)	97 (12.5)	59 (8.6)
BDE 183	59 (8.3)	60 (2.7)	62 (7.9)	53 (12.7)	126 (0.1)	35 (3.5)
BDE 209	52 (23.8)	96 (0.1)	65 (10.2)	169 (13.3)	90 (12.8)	122 (6.2)
AVERAGE	66.88	63.88	63.13	75.88	89.38	54.13
SD	11.46	14.40	9.00	39.66	17.44	28.69

Further recovery studies were carried out on effluents with the Isolute PAH cartridge at two fortification levels; 3 ng/L and 10 ng/L for all BDEs, except BDE 209, which was studied at 30 ng/L and 100 ng/L due to its relatively higher presence in wastewater. Recoveries were surrogate standard-corrected using PCB 209 and 4PC-BDE-208. The US EPA method 1614 recommends analyte recovery of 60 – 140 % for BDE 28 – 183, and 50 – 200% for BDE 209 with an RSD less than 40 % for initial demonstration of method precision and accuracy (USEPA, 2007). At 3 ng/L fortification level (Table 3-13), good recoveries (78- 135%) were recorded for all analytes except BDE 47 (176%). Recoveries of PBDEs at 10 ng/L were mostly between the acceptable ranges of 60 – 129 %, except BDE 183 with recovery of 150 % (Table 3-13). RSDs were generally lower than 20% thereby showing method precision.

The MDLs were determined with a spike concentration of 0.5 ng/L (5 ng/L for BDE 209 and surrogate- 4PC-BDE-208) and 1 ng/L (10 ng/L for BDE 209 and surrogate- 4PC-BDE-208) in DI water and effluent respectively. As shown in Table 3-13, the MDL of the eight PBDE congeners in water samples ranged from 0.14 ng/L to 10 ng/L in deionized water and 0.2 ng/L to 10.8 ng/L in effluent samples. These determined MDLs passed the acceptance criteria in Section 3.2.2.5 (i.e. MDL < spike concentration or ≤ 5 times spike concentration, and ≥ spiked concentration/10). Furthermore, these values are below the reported levels of these chemicals in wastewater around the world (Peng *et al.*, 2009; Kim *et al.*, 2013; Deng *et al.*, 2015), hence the method can be used to quantify them accurately. The method detection limits achieved was also below the maximum allowable concentration environmental quality standards (MAC-EQSs; 140 ng/L and 14 ng/L for inland and other surface waters

respectively) for PBDEs (sum of congeners BDEs 28, 47, 99, 100, 153 and 154) as proposed in the EU Water Framework Directive (EU, 2013a).

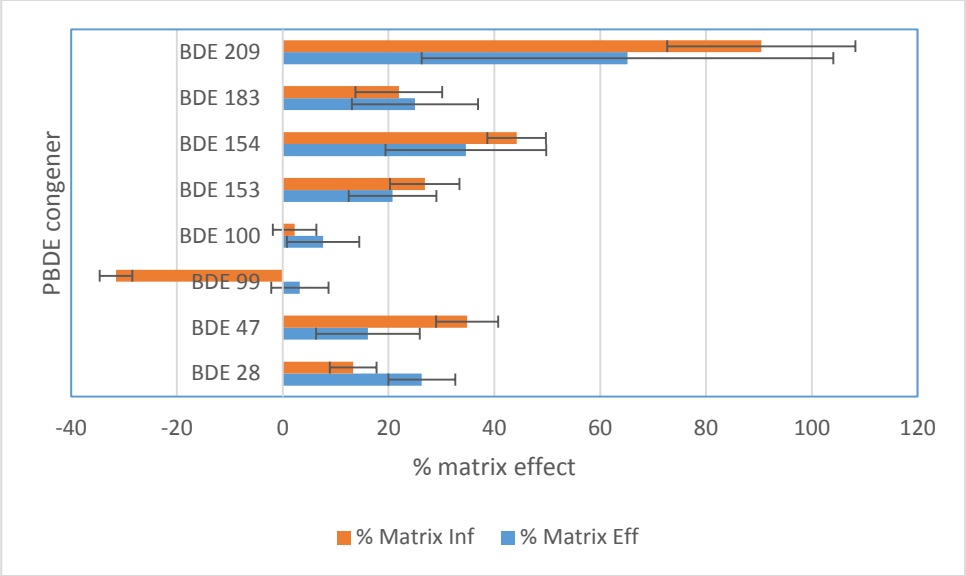


Figure 3-6 Observed matrix effect in the analysis of PBDEs in influent and effluent samples.

Table 3-13 Surrogate corrected recovery at 3 ng/L and 10 ng/L in effluent, instrumental detection limit (IDL) and method detection limit of PBDE congeners in deionized (DI) water and effluent. Surrogate standards are indicated in italics.

Chemical	Corrected recovery in effluent (%)		IDL (pg/µl)	MDL (ng/L)	
	3 ng/L	10 ng/L		DI water	Effluent
BDE 28	78 (16.1)	90 (7.0)	0.2	0.68	0.66
BDE 47	176 (11.7)	129 (15.2)	0.2	0.54	2.57
BDE 99	74 (6.4)	116 (4.7)	0.5	0.33	2.54
BDE 100	62 (5.1)	83 (7.3)	0.5	0.27	1.89
BDE 153	78 (1.8)	112 (9.1)	0.5	0.14	0.20
BDE 154	131 (7.5)	60 (6.7)	0.5	0.44	4.19
BDE 183	86 (8.2)	150 (6.7)	1.0	0.53	1.31
BDE 209	135 (3.4)	113 (2.3)	5.0	10.04	10.76
PCB 209	-	-	0.2	0.28	1.09
4PC-BDE-208	-	-	5.0	4.37	6.12

3.2.4 **Application to real wastewater samples**

The developed method was employed to investigate the concentrations of eight primary PBDE congeners in both the dissolved and particulate matter phase of the wastewater, and assess the mass removal rate achieved by the treatment plant. The PBDE congener profile in influent and effluent showed the presence of seven congeners; BDE 100 was not detected (Table 3-14). The total (aqueous and particulate matter) concentration of each PBDE was between 2.1 – 111 ng/L (∑PBDE = 169 ng/L) in raw influent and 1.6 – 17.4 ng/L (∑PBDE = 43 ng/L, ∑PBDE EU WFD congeners = 19 ng/L) in final effluent (Table 3-14). These concentrations are an order of magnitude lower than the EU WFD MAC-EQS and are similar to reported levels in Canada (Kim *et al.*, 2013) Australia (Clarke *et al.*, 2010), and China (Deng *et al.*, 2015). Risk assessment for BDE 209 was performed as reported by Cristale *et al.*, (Cristale *et al.*, 2013). The predicted no effect concentration (PNEC) for aquatic organisms (including fishes, dapnids and algae was calculated as 4.82) and maximum measured concentration (MC) was used to obtain a risk quotient (RQ = MC/PNEC). Low to significant adverse effect of BDE 209 was indicated when $1.0 \leq RQ < 10$, and $10 \leq RQ < 100$ respectively. In the present study, low potential for adverse effects on aquatic organisms was observed for BDE 209 in effluent of the WWTP, with an RQ of 3.7.

Table 3-14 Concentration of BDE congeners in effluent and influent of a UK activated sludge WWTP.

PBDE congener	Influent (ng/L)	Effluent (ng/L)	% Removal
BDE 28¶	11.0 (1.3)	4.4 (0.2)	60.0
BDE 47¶	7.1 (0.2)	6.0 (0.1)	15.5
BDE 99¶	10.7 (0.4)	2.8 (0.8)	73.8
BDE 100¶	< 1.89	< 1.89	-
BDE 153¶	7.1 (0.6)	1.6 (0.3)	77.5
BDE 154¶	2.1 (0.3)	4.0 (1.3)	0
BDE 183	20.0 (0.2)	6.3 (0.5)	68.5
BDE 209	111.3 (10.7)	17.7 (1.4)	84.1
∑PBDE	169.4	42.9	74.7
∑PBDE EU MAC-EQS	-	18.8	29.9*

¶ *PBDE congeners included in the EU WFD * Average % removal*

The concentration of BDE 209 represented 66% of the total PBDE concentration in influent and 40% in effluent samples (Figure 3-7). BDE 209 has been reported to dominate the total concentration of PBDE found in wastewater around the world (Peng *et al.*, 2009; Kim *et al.*, 2013; Wang *et al.*, 2013c). Furthermore, only BDE 209 was detected in a river receiving effluent from wastewater treatment plants in the United Kingdom, to a concentration of up to 290 ng/L which were associated with significant levels of risk to aquatic wildlife (Cristale *et al.*, 2013). About 82% and 49 % of PBDE was present in the particulate phases of the influent and effluent respectively (Figure 3-8). This observation is as expected due to the high log K_{OC} values (Table 3-9) of the PBDE congeners, which give rise to a high association with suspended solids. This observation is in line with previous findings that over 90% of PBDEs in influent tends to absorb to sludge in WWTP (North, 2004; Song *et al.*, 2006). About 75% removal of total PBDE concentration was achieved by the WWTP; this removal is most likely due to partitioning and settling out with the sludge in the primary and secondary sedimentary tanks (Song *et al.*, 2006). Concentrations in sludge are reported to vary over one order of magnitude (Hale *et al.*, 2003; Lee *et al.*, 2014), while their concentrations in anaerobically treated sludge are unknown. The potential risks PBDEs pose via sludge applications to soil is therefore also unknown. Furthermore, the difference in the distribution of PBDE between the dissolved and particulate matter (PM) in influent and effluent samples (Figure 3-8) indicates that PBDEs was mostly removed via the PM phase throughout the WWTP. It must be noted that wastewater treatment is the preserve of high-income countries and cities, with 80% of the worlds' wastewater going untreated into receiving watercourses (UNESCO, 2012). Concentrations of PBDEs may be at levels that cause a risk to aquatic wildlife in highly populated urban centres, especially in low to middle income countries.

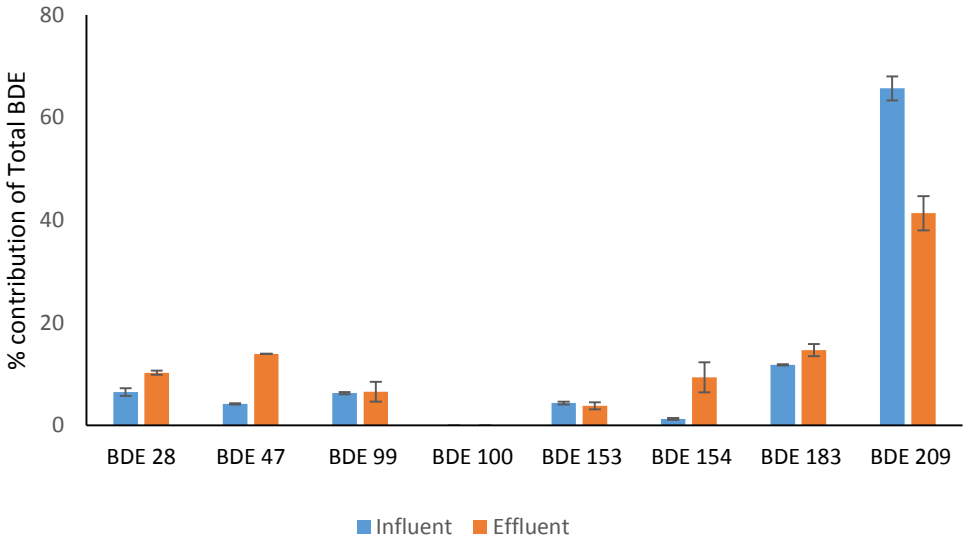


Figure 3-7 Distribution of BDE congeners in influent and effluent samples from a UK WWTP.

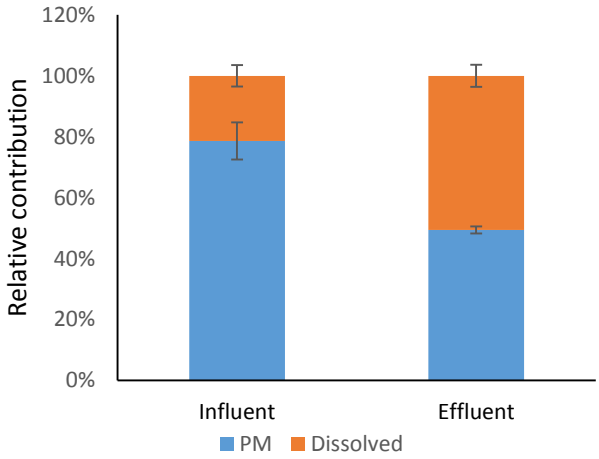


Figure 3-8 Partitioning of PBDEs over particulate matter (PM) and dissolved phases of influent and effluent samples from a UK wastewater treatment plant.

3.2.6 **Conclusion**

In this study, a novel SPE-GC-ECD method was developed for the analysis of eight primary PBDE congeners in wastewater. The extraction procedure was optimized by testing different SPE cartridges including Oasis HLB, Isolute C18 and Isolute PAH. The Isolute PAH cartridge proved to be superior in sample clean up and extracting low and high molecular weight PBDEs in wastewater, perhaps due to its unique combination of a C18 and amino based sorbent. Furthermore, the chromatographic performance was optimized, thereby allowing determination of BDE 28 to BDE 209 in a single run- in contrast to previously reported GC-ECD methods. The detection of the PBDEs by this method was corroborated using GC-MS. The low detection limits obtained allowed for determination of PBDEs at environmentally relevant levels, and, importantly, were well below the proposed MAC-EQSs for PBDEs set by the EU Water Framework Directive. Although the method presented here may have limitations due to a lack of selectivity of the GC-ECD compared to a GC-MS/MS system, it is a more cost-effective solution for quantifying PBDE concentrations in wastewater. GC-ECD is two-fold cheaper than GC-MS in capital costs and up to four-fold cheaper in operational costs since all PBDE congeners can be analysed on a single run. This method thereby opens up PBDE analysis to more laboratories without a GC-MS system or can allow them to commit GC-MS systems to other functions. This will be more useful in developing countries with limited resources to carry out environmental analysis. Hence, if we rely solely on the best methods, we would miss the broader picture of a global fight against chemical pollution; which of-course starts with the ability to detect and measure these chemicals in the water.

The novel method was employed for the quantification of PBDEs at trace levels in the influent and effluent of a UK conventional activated sludge WWTP. All BDEs of interest were detected in both influent and effluent samples at trace concentrations, that is, in the 2-20 ng/L range, except BDE 100, which was present at about tenfold higher concentrations. BDE 209 was the most abundant analyte, and 50 – 80% of the BDEs partitioned onto the particulate matter. Finally, about 75% removal of the total PBDE concentration was achieved by the WWTP to levels assessed to pose little risk to aquatic wildlife. However, PBDE removal was most likely due to adsorption onto sludge during secondary treatment. Urban areas with little or no wastewater treatment might not be afforded the same level of environmental protection against PBDEs offered by the WWTP in this study area.

Chapter 4: UK Biodegradation Studies

Chapter 4 Biodegradation of triclosan, 15 priority PAHs and estrogens under aerobic and anaerobic wastewater treatment conditions (UK studies)

4.1 Introduction

Emerging contaminants such as natural/synthetic estrogens (hormones), triclosan (personal care product) and polyaromatic hydrocarbons (industrial chemical) have received increasing attention within the last two decades due to their adverse effects, which includes toxicity, bioaccumulation and endocrine disruption (Roh *et al.*, 2009; Combalbert and Hernandez-Raquet, 2010; Liu *et al.*, 2015). Aside being toxic to aquatic organisms such as algae, dapnids and fishes (Singer *et al.*, 2002; Kookana *et al.*, 2011), triclosan has also been reported to exhibit estrogenic activities in rats and fish (Ishibashi *et al.*, 2004; Feng *et al.*, 2016). Polyaromatic hydrocarbons (PAHs) are also a significant group suspected to exhibit estrogenic activities (Kummer *et al.*, 2008), though they are best known for their mutagenic and carcinogenic activities (Jones *et al.*, 2012). Strong estrogenicity has been reported for natural estrogens estrone (E1), 17β-estradiol, estriol (E3) and the synthetic estrogen (EE2) (Combalbert and Hernandez-Raquet, 2010; Liu *et al.*, 2010). The widespread use of such chemicals eventually results in their introduction in wastewater either through domestic use or run-off into the combined sewer network in the UK. WWTPs therefore act as a conduit for such pollutants but could also be an important point for their control. Hence, their effective removal in WWTPs is imperative to protect receiving water bodies.

A review on the occurrence of these pollutants in wastewater (Deblonde *et al.*, 2011) has been published, as well as their fate and removal in WWTPs by physical, chemical and biological processes (Liu *et al.*, 2009; Verlicchi *et al.*, 2012). Sorption and biodegradation have been identified as the key mechanisms for the removal of these pollutants in WWTPs; and the extent of biodegradation is affected by key operational parameters such as temperature, hydraulic retention time (HRT) and solid retention time (SRT) (Alvarino *et al.*, 2014). Biodegradation, either by deconjugation, cometabolism with nitrifying biomass or heterotrophic bacteria has been attributed to estrogen, triclosan and PAHs removal in WWTPs after initial sorption (Ren *et al.*, 2007; Haritash and Kaushik, 2009; Roh *et al.*, 2009; Racz and Goel, 2010). Furthermore, removal pathways and degradation efficiencies of these chemicals in WWTPs is dependent on the applied redox potential- aerobic, anoxic or anaerobic (Joss *et al.*, 2004; Xue *et al.*, 2010). A review by Liu *et al*. shows that several studies have compared the fate of estrogens under relevant aerobic and anaerobic conditions wastewater treatment- (Liu *et al.*, 2015). On the other hand, while the fate of triclosan under aerobic conditions is

sufficiently documented, there is limited information on its fate under anaerobic conditions (Chen *et al.*, 2011). Most studies on PAH biodegradation have focused on the anaerobic and aerobic digestion of sewage sludge rather than the main wastewater treatment processes (Trably *et al.*, 2005; Cea-Barcia *et al.*, 2013).

The water industry is under regulatory pressure to improve the removal of such pollutants, at a time when the sustainability of the current energy intensive treatment systems (such as activated sludge) which accounts for up to 1.5 % of the UK electricity use is under scrutiny (Li *et al.*, 2015; Water-UK, 2017). Consequentially, anaerobic-aerobic hybrid units are gaining popularity because of significant advantages including lower aeration energy requirement, production of methane for biogas and lower sludge generation (Buntner *et al.*, 2011). Knowing the extent of degradation of these compounds in aerobic and anaerobic systems is required to understand the limits of engineering biological systems (e.g. required HRT) for the removal of such chemicals (Smith *et al.*, 2015a; Petropoulos *et al.*, 2017b). From the up to date literature, it is known that current wastewater systems utilizing aerobic or anaerobic technologies fall short in removing these chemicals below the required limits. Since biodegradation has been identified as a potential major removal mechanism for micropollutants, it is imperative to investigate degradation of these compounds under different redox conditions and at operational relevant temperatures in the UK- as this critical information can be used to predict effluent concentrations.

The purpose of this study was to carry out biodegradation experiments for triclosan, four estrogens and fifteen PAHs under aerobic and anaerobic conditions using inocula from different wastewater treatment processes in the UK including those using different strategies (aerobic activated sludge, a mesophilic low-temperature-adapted inocula, and an alpine-arctic low temperature-adapted anaerobic inocula) towards a sustainable wastewater treatment. Furthermore, the effect of chemical structure and functional groups on the degradation of these chemicals was investigated under different redox conditions by comparing their degradation kinetics. The influence of sorption on the disappearance of the chemicals was also evaluated. Microbiological properties of the inocula were characterized, and the change in bacterial taxa and genera suspected to be responsible PAHs degradation at the end of the biodegradation assay were identified.

4.2 Experimental

4.2.1 Materials

Details on analytical standards, solvents, filters and solid phase extraction cartridges for triclosan and PAH analysis are given in Section 3.1.2 & 3.2.2. Ultra-trace grade of MTBE and HPLC water for estrogen analysis were obtained from Sigma Aldrich (UK), while the other materials used were mentioned by (Coello-Garcia, 2018) (submitted).

4.2.2 Sample collection and storage

Activated sludge grab samples for the experiments were collected from the aeration basin of a municipal wastewater treatment plant (WWTP) located in North East, England. The WWTP was a nitrifying treatment plant that receives 6751 m^3/day of domestic wastewater with a population equivalent of 22,500 people. Samples were collected in cleaned and disinfected (with 1% Virkron for 24 hours, followed by several rinse cycles with distilled water) 5 litres high density polyethylene (HDPE) containers - analysis of the containers showed that they were not contaminated with any of the target compounds. Samples were stored at 4 ^oC upon arrival to the laboratory and were processed within 48 hours. The total suspended solids (TSS) and volatile suspended solids (VSS) content of the sludge were measured according to the Standard Method 2540B (Clesceri *et al.*, 2005).The measured values of TSS and VSS were 3.3 g/L and 2.3 g/L respectively. Activated sludge grab samples used in the aerobic biodegradation experiments of estrogens were carried out by (Coello-Garcia, 2018) and were collected from a WWTP in North East England. This WWTP has a population equivalent of 28,800 and the TSS and VSS content of the sludge was 1.92 g/L and 1.54 g/L respectively.

Anaerobic sludge grab samples were collected from a pilot scale UASB plant located at Cranfield University, United Kingdom. This UASB reactor has a capacity of 70 L and operated with a daily flow and hydraulic retention time of 210 L and 8 h respectively (Garcia *et al.*, 2013). The sludge was a mesophilic UASB sludge adapted to ambient domestic wastewater and its TSS and VSS content was 26.1 g/L and 20.4 g/L respectively. The sludge was collected from a fully functional UASB plant, and was immediately transported to Newcastle where it was stored at 4^oC and processed within 48 hours. The sampling materials and procedures for this UASB sludge was similar to those applied for activated sludge samples.

Inocula used for the low temperature anaerobic experiment was from Petropolous *et al*., (Petropoulos *et al.*, 2017a), and was an equal mixture of cold-adapted sediment from Lake

Geneva ‘’N 46°23′04″, E 6°25′07″ (average temperature – 11 to - 17 ° C) and soils from Svalbard, in the high Arctic at various sampling points situated at ‘’N78°, E11, 15,16°’’ (average temperature -16–6 °C) that had been adapted for the treatment of wastewater at 4 – 15ºC for 100 days . The TSS and VSS content was 26.2 g/L and 3.8 g/L respectively.

4.2.3 **Experimental design of biodegradation assay**

Batch reactors were used for the degradation experiments as they have previously been used to mimic biotransformation reactions and kinetics in a full scale WWTP (Helbling *et al.*, 2012). Aerobic biodegradation experiments were carried out in 1 L amber glass bottles (test vessels) (Figure 4-1). 500 ml of sludge was added to the test vessels followed by addition of the chemicals (1000 µg/L for triclosan, 200 µg /L for PAHs) in a solution of methanol (for triclosan) or acetone (for PAHs)- volume of solvent added was limited to 0.1% to minimize any potential effect). These solvents will not inhibit microbial action as methanol is non-toxic to microorganisms below 1000 mg/L (Novak *et al.*, 1985; Brasil Bernardelli *et al.*, 2015) and acetone is non-toxic below 5 % v/v in different redox conditions (González, 2006). The experiment for triclosan and PAHs were performed individually and in separate test vessels. Reactors were placed in an incubator equipped with a shaker (IKA KS 4000), and set at 140 rpm to ensure homogenous mixing and aeration of the sludge. The experiment was performed at 20 ºC in a temperature controlled room. Dissolved oxygen and pH were monitored throughout the experiment to ensure that these parameters were not limiting, and they ranged from 4 – 7 mg/L and pH of 5 – 7 respectively- especially, as pH of the sludge has been reported to affect the adsorption of triclosan (Lindström *et al.*, 2002). The estrogen experiment was carried out by (Coello-Garcia, 2018) by a slightly different procedure. The major difference was the incubation temperature (15 ºC) and the spiking method (chemicals were spiked into reactor in methanol, which was then allowed to evaporate in a fume hood before the addition of activated sludge). All other parameters and procedures were similar. For extraction of the chemicals, triplicate samples (1 ml per sample) were collected in 2 ml Eeppendorf tubes, spiked with surrogate standards (isotope labelled triclosan, PAHs and estrogens standard solutions) and immediately passed through preconditioned solid phase extraction (SPE) cartridges. The cartridges were washed with appropriate solvents (see Section 3.1; Coello-Garcia, 2018), dried for 20 – 30 minutes, covered with top caps and stored at -20 ºC. Samples were acidified to pH 2 with sulphuric acid for triclosan analysis only, before processing with SPE. SPE extraction of triclosan was better at pH 2 as mentioned in Section 3.1.3.

The batch test vessels were sampled at multiple points (0, 3, 24, 48, 72, 96, 120, 144, and 168 hours) for a duration of 168 hours for triclosan and PAHs, and 72 hours (0, 0.5, 0.7, 1, 2, 4, 8, 12, 24, 48, and 72 hours) for estrogens. After the initial addition of activated sludge, the test vessels were not fed with wastewater or any additional media for the duration of the experiment. 2 mL samples were also taken from the reactors to investigate degradation in the aqueous and particulate matter phase individually, and estimate any potential losses due to adsorption. These samples were centrifuged (16,437 x g, 2 min) and 1 mL of the supernatant was processed as described for the samples above.

An inhibition and abiotic control was employed to check losses due to non-biological degradation; hydrolysis or volatilization. 500 ml of activated sludge in a test vessel was inactivated by autoclaving twice (24 hours apart) at 121 ^{o}C and 103 kPa for 20 minutes as described by Helbling *et al*., (Helbling *et al.*, 2010). The chemicals were dissolved into methanol or acetone, then spiked into these control test vessels at the same concentrations as the live test vessels. Triplicate samples (1 ml) were taken and processed as described previously. All test vessels were initially sterilized by autoclaving (at 121 ^{o}C and 103 kPa for 20 minutes) before addition of sludge and compounds.

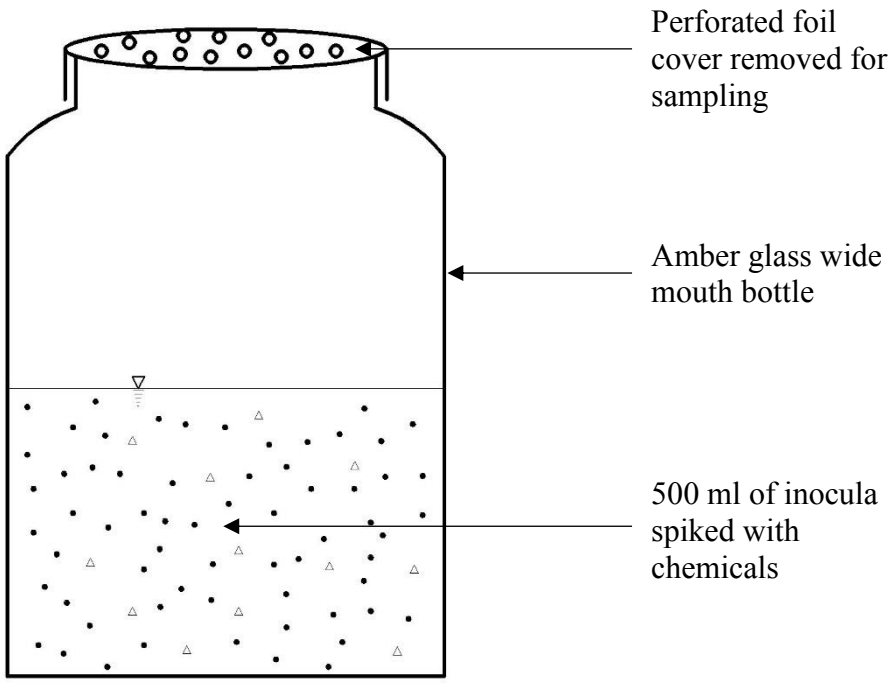


Figure 4-1 Schematic diagram of aerobic biodegradation test vessel

Anaerobic biodegradation experiments were carried out in 500 ml clear glass serum bottles with 30 mm crimp tops. 250 ml of UASB or low temperature anaerobic inocula was added to the test vessels and spiked with 3000 µg/L of triclosan, 600 µg/L of PAHs and 300 µg/L for estrogens. These compounds were spiked into the test vessels in a solution of methanol or acetone (limited to 0.2 % v/v of reactor). Test vessels were sealed with a 30-mm crimp cap with chlorobutyl vial stoppers and purged with nitrogen gas for 20 minutes to enable anaerobic incubation conditions. The test vessels were covered completely with aluminium foil to prevent photolytic degradation and were placed in an incubator at 20 ^{o}C. Samples (0.2 ml) were taken from the reactor using a 2 ml sterile disposable syringe with a 20 G needle (VWR, UK) into 2 ml Eppendorf tubes. Surrogate standards were immediately added (at 100 ng/ml, 200 ng/ml and 50 ng/ml for triclosan, PAHs and estrogens respectively to the samples and were transferred into preconditioned SPE cartridges for extraction and were processed similar to the aerobic experiments (samples for triclosan analysis were acidified to pH 2 as mentioned above). The experiment was incubated for 28 days and sampled at multiple points (sampling at day 0, and after every subsequent 3 days). After the initial addition of the UASB sludge, the test vessels were not fed with any media (carbon source) until the end of the experiment. Samples (0.3 mL) were collected to investigate biotransformation in both aqueous and solid phase and were processed similar to the aerobic experiment. Inactivated control (autoclaved sludge to check abiotic losses) were also monitored similar to the aerobic experiment, except 0.3 mL sample was used. 250 mL of the anaerobic sludge were inhibited by autoclaving twice as previously described in the aerobic experiment. The test vessels were also initially sterilized by autoclaving before use.

4.2.4 **Extraction and instrumental analysis**

The extraction of triclosan and PAHs using SPE was previously described in Section 3.1.2, while the SPE based extraction of estrogens was adopted from (Coello-Garcia, 2018). Briefly, 0.2 – 1 mL of sludge was passed through preconditioned SPE cartridges (water + methanol for triclosan, water + isopropanol for PAHs, water + methanol + mtbe for estrogens), washed with diluted organic solutions, dried and stored. Elution was performed with ethyl acetate, dichloromethane (DCM), and 0. 5 % ammonia in methanol for triclosan, PAHs and estrogens respectively. This was followed by evaporation with nitrogen, and reconstitution to 1 mL with DCM for triclosan and PAHs, and 5 % acetonitrile for estrogens in a 2 mL glass vial. Triclosan samples were derivatized with 100 µl of BSTFA with 1% TCMS before instrumental analysis. Instrumental methods from Section 3.1.2 using gas chromatography coupled with a mass spectrometer was adopted for triclosan and PAHs analysis. Isotope

dilution quantification was employed, and the multipoint calibration curve ranged from 20 – 5000 ng/ml for triclosan, 2 – 500 ng/ml for methyl triclosan and PAHs. Isotope labelled triclosan and four PAHs were used as surrogate standards. Estrogen extracts from the sludge samples were analysed by liquid chromatography coupled with a triple quadrupole mass detector, according to method conditions reported by (Coello-Garcia, 2018). Isotope dilution quantification was employed, and the multipoint calibration curve ranged from 5 – 1000 ng/ml.

4.2.5 Preliminary tests and method validation

4.2.5.1 Quality assurance

Full method validation data for triclosan and PAHs were described in Section 3.2.3, while those for estrogens were described by (Coello-Garcia, 2018). Additionally recovery studies were used to validate the analysis of the chemicals from biosolids (sludge). These recovery studies were carried out by dosing 500 mL of activated sludge with triclosan, and PAHs at 1000 µg/L and 200 µg/L respectively. Samples were taken following similar procedures described above, and the relative ratio of analyte to surrogate was compared in the samples to that of an analytical standard. Furthermore, to ensure homogeneity in the distribution of the chemicals in the bioreactor before sampling, a solubility test for triclosan and PAHs in wastewater was carried out. This was done by spiking 500 mL of freshly collected activated sludge with the chemicals at 100 µg/L in a bioreactor, sampling at 1 min, 5 min and 15 min, and processing these through the SPE-GC-MS system.

4.2.5.2 Phase partitioning test

An experiment was carried out to determine the partitioning of the chemicals onto the aqueous and solid phases of sludge samples. Two methods were employed and compared for this test, while triclosan and activated sludge was selected as the test chemical and sludge type respectively. Triclosan was selected as the test chemical as volatilization at room temperature was highly unlikely due to its low vapour pressure of 0.00062 Pa (Bester, 2005). Therefore, it will exist in either the aqueous or solid phase. The first method (Method 1) involved centrifuging a 1.2 ml sample taken out of a test reactor (500 ml of activated sludge spiked with 1000 µg/L of triclosan) at 16,437 x g for 2 minutes. 1 ml of the supernatant was then spiked with label triclosan then passed through preconditioned C_{18} SPE cartridge and processed according to the procedures mentioned above. The relative response (RR, calculated by dividing the peak area of the compound by that of the surrogate) obtained was then compared to that of an analytical standard (at the spiked concentration), and the

difference was assumed to be retained in the solid phase. Labelled triclosan was also spiked before centrifugation to allow for surrogate standard correction.

The second method (Method 2) involved the use of depth filter purchased from Biotage (UK). These depth filters are similar to the SPE cartridges, but differ in the internal packing. They are packed with a foam-like material to help remove solids from water samples, and are usual mounted on an SPE cartridge. The depth filter was conditioned with 5 ml of deionized (DI) water, then placed on a preconditioned C_{18} SPE cartridge using a column adaptor. 1 ml was taken from the test reactor (as above), and passed through the depth filter under vacuum. The depth filter was then washed with 5 ml of DI water, so that all the liquid phase samples are collected in the attached SPE cartridge and the solids are retained in the depth filter. The depth filter was detached and connected to a new SPE cartridge. Elution was performed by passing 8 ml (2 x 4 ml) of methanol through the depth filter. Triclosan in the liquid phase was also eluted with 8 ml of methanol from the previously attached SPE cartridge. The extracts were analysed following procedures mentioned previously. The peak area of both the liquid and solid samples were compared to that of an analytical standard to see the possible effect of matrix. Labelled triclosan was also spiked in the test reactor and processed together with parent triclosan.

4.3 Microbial analysis

4.3.1 DNA extraction

Genomic DNA (gDNA) was extracted from the 1 ml of inoculum sampled during the biodegradation experiment (at the beginning and end) using FastDNA Spin Kit for Soil according to the manufacturer's protocol (MPBiomedicals Santa Ana, CA, USA). The extracted DNA was stored at -20 ^{o}C until further use.

4.3.2 PCR amplicon library preparation

A fusion PCR method was used to generate the amplicon library for Ion Torrent sequencing. The fusion primers consists of Ion Torrent adaptor sequence and primer sequence. The forward primer contains a unique barcode sequence that differentiates the sequence corresponding to each sample during analysis. The samples were labelled with a unique 12 bp Golay barcode, added to the 5' end of the forward primer through a GAT spacer, and attached to the Ion Torrent adapter A (5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3'). The reverse primers however were attached to the Ion Torrent adapter trP1 (5'-CCTCTCTATGGGCAGTCGGTGAT-3').The primer set [515f (5'-

GTGNCAGCMGCCGCGGTAA-3') and 926r (5'-CCGYCAATTYMTTTRAGTTT-3')] employed, amplified the V4 and V5 regions of 16S rRNA gene (Parada *et al.*, 2016). The PCR reaction was performed using Phusion Flash High-Fidelity PCR Master Mix Kit (Thermo Scientific, UK) according to the manufacturer's instructions. A 20 µL reaction solution containing 10 µL of Phusion Flash Master Mix, 0.5 µM of each universal primer, 1 µL of DNA template (or sterile nuclease-free molecular grade water for negative control) and molecular water. The thermocycler program used for amplifications involves an initial denaturation cycle of 98 ^{o}C for 10 seconds followed by 35 cycles of denaturation (at 98 ^{o}C for 1 second), annealing for 5 seconds (at 56 ^{o}C) and elongation for 15 seconds (at 72 ^{o}C). This was followed by a final extension step for 1 minute (at 72 ^{o}C). The PCR products were stored at -20^{o}C until further use.

The PCR products were cleaned and size selected using a double sided solid-phase reversible immobilisation (SPRI) beads (Agencourt AMPure XP system, Beckman Coulter, UK) following the manufacturer's instructions. These PCR amplicons were then quantified using a Qubit® 2.0 Fluorometer according to the manufacturer's instructions and the samples were pooled in equimolar concentrations. This was followed by sequencing on a Personal Genome Machine (PGM) using a 316 ion chip in the Environmental Engineering Labs at Newcastle University. This sequencing was carried out by Dan Curtis and Amy Bell.

4.3.3 **Sequencing Data Analysis**

The sequences were processed using the Quantitative Insight Into Microbial Ecology (QIIME) 1.9.1 pipeline using default parameters (Caporaso *et al.*, 2010). The reads obtained after sequencing were filtered to match the sequence barcode and a minimum sequencing length of 200 bp using QIIME Deionizer (Reeder and Knight, 2010). The QIIME pipline processing briefly consisted of binning sequences into Operational Taxonominc Units (OTUs) using a 97 % identity threshold, and for each OTU, the most abundance sequence was selected to represent them. Taxonomy was assigned to the bacterial OTUs from a Greengenes database subset (http://greengenes.lbl.gov/) (DeSantis *et al.*, 2006). UCLUST was then used to align representative OTU sequences. The number of bacterial 16S rRNA gene sequences per sample was between 19,671 – 114,361; summing up to a total number of reads of 268,753 sequences. Sequences were pruned to an even depth (5000 per sample- based on the sample with the lowest number of reads) for downstream analysis as recommended for alpha and beta diversity comparison (Shaw *et al.*, 2008). The Bray Curtis dissimilarity metric was calculated

for OTU table at genus taxanomy level and visualized using 2 dimensional non-metric multidimensional scaling plot (NMDS) using R (Clarke and Warwick, 2001).

4.4 Statistical analysis

Minitab, version 17 Statistical software (Minitab Inc., USA) was used to perform all statistical analysis. Variance analysis using one way ANOVA was used to compare the biodegradation rates of the investigated chemicals under the applied redox conditions and inoculum type. One-way ANOVA was also employed to compare the alpha diversity indices in a specific inoculum (activated sludge or UASB) spiked with the selected chemicals at the beginning and end of the biodegradation experiment. Furthermore, STAMP was used to identify the change in bacterial community taxonomy before and after the aerobic or anaerobic treatment (Parks *et al.*, 2014). A two-sided G-test (w/ Yates') + Fisher's statistical test was carried in STAMP on each pair of sample to determine any significantly difference ($p < 0.05$) in the bacteria taxa.

4.5 Results and discussion

This section first discusses the results of the quality assurance test for the analysis of triclosan and PAHs in activated sludge. This was followed by results from the phase partitioning test for triclosan. Finally, the results of the degradation study under aerobic and anaerobic conditions were presented and discussed.

4.5.1 Quality assurance

The recovery rate of triclosan in activated sludge was 101 % with an RSD of 2.7 % (Table 4-1), and was similar to that obtained for wastewater effluent in Section 3.1.3. The recoveries of PAHs ranged from 67 – 107 % with an RSD of between 0.8 – 11 % (Table 4-1). These good recoveries and RSDs demonstrates method accuracy and precision.

Table 4-1 Analytical methods and recovery of triclosan, PAHs and estrogens from sludge

Compound	Analytical method	Quantification ion (m/z)	Relative recovery (RR) (%)	Relative standard deviation (RSD)
Triclosan	GC-MS	347	100.5	2.7
Naphthalene	GC-MS	128	82.1	5.0
Acenaphthylene	GC-MS	152	71.1	7.3
Acenaphthene	GC-MS	153	82.9	8.1
Fluorene	GC-MS	166	89.1	4.9
Phenanthrene	GC-MS	178	83.1	3.5
Anthracene	GC-MS	178	78.5	0.8
Fluoranthene	GC-MS	202	97.8	2.4
Pyrene	GC-MS	202	96.8	2.0
Benz(a) anthracene	GC-MS	228	83.9	1.6
Chrysene	GC-MS	228	87.7	3.7
Benzo(b) fluoranthene	GC-MS	252	82.9	4.2
Benzo(a)pyrene	GC-MS	252	86.9	5.8
Indeno (1,2,3-cd)pyrene	GC-MS	276	88.2	10.8
Dibenz(a,h) anthracene	GC-MS	278	107.2	7.0
Benzo(ghi) perylene	GC-MS	276	66.9	10.4

Additionally, the result of a solubility test showed (data not shown) that the concentration of triclosan and PAHs remained the same from 1 min to 15 min, indicating fast homogeneity and solubility. Hence, the first sample from the reactor was taken after 10 minutes.

4.5.2 **Phase partitioning test**

The result of the phase partitioning test showed that triclosan partitions mostly onto solids, and this is expected due to its high log K_{ow} value of 4.8 (Lindström *et al.*, 2002; Bester, 2005). Results from Method 1 (centrifugation) showed that 96.5 % of triclosan was sorbed onto the solids, while 3.5% remained in the dissolved phase (Table 4-2). Similar result shows that 93.3 % and 3.8 % of triclosan was present in the solid and dissolved phase respectively when Method 2 (depth filter) was applied (Table 4-3). These results support findings that over 90 % of triclosan in activated sludge was sorbed onto the solids throughout the degradation experiment carried out by Chen *et al*., (Chen *et al.*, 2011). Since, the results of the two methods were similar, Method 1 (centrifugation) was adopted to monitor adsorption of triclosan and other investigated chemicals in the degradation experiments. This method was easier, less time consuming and allowed for isotope dilution quantification of the chemicals.

Table 4-2 Partitioning of triclosan using centrifugation (Method 1) for phase separation

Compound	RR of standard	RR sample with pre-centrifugation surrogate addition	RR sample with post-centrifugation surrogate addition
Triclosan	8.5 (2.3)	9.2 (0.8)	0.3 (4.3)
	Solid phase		Dissolved phase
% triclosan in phase	96.5		3.5

RR represents relative response

Table 4-3 Partitioning of triclosan using depth filter (Method 2) for phase separation

Compound	Concentration of standard (ng/L)	Solid phase concentration (ng/L)	Dissolved phase concentration (ng/L)
	100 (0.8)	93.3 (3.9)	3.8 (0.2)
% triclosan in phase		93.3	3.8

4.5.3 Degradation of triclosan under aerobic and anaerobic conditions

4.5.3.1 Biotransformation under aerobic condition

No sign of non-biological degradation (chemical degradation or photo-degradation) or abiotic losses (such as volatilization and hydrolysis) was observed as the concentration of triclosan remained constant in the inactivated sludge control throughout the experiment (Figure 4-2). Hence, the losses of triclosan observed in the experiment were due to biodegradation. Triclosan was reduced from an initial concentration of 806 µg/L (1000 µg/L was spiked) to a final concentration of 234 µg/L in 168 hours (75 % removal) (Figure 4-2, Figure 4-3,Table 4-4). Chen *et al.*, reported a similar observation of 86 % degradation of triclosan spiked at 500 µg/L after 168 hours (Chen *et al.*, 2011). Furthermore, results of the analysis of triclosan in the aqueous phase, showed that 95 – 98 % of triclosan was sorbed to the activated sludge solids throughout the experiment.

The production of methyl triclosan was observed in the bioreactors, and its concentration increased with decreasing triclosan concentration (Figure 4-3). Methyl triclosan is known to be more persistent, bio-accumulative and lipophilic than triclosan (Lindström *et al.*, 2002; Balmer *et al.*, 2004). The concentration of methyl triclosan increased from 1 µg/L to 17.3 µg/L after 144 hours (Figure 4-3). About 1.6 % of the triclosan in this reactor was converted to methyl triclosan, thereby supporting reports that methylation is a possible biotransformation pathway for triclosan under aerobic conditions (Heidler and Halden, 2007). However, the 1.6 % methylation rate suggests other major primary transformation product(s) formed through (a) different biotransformation pathway(s) under aerobic conditions. This observed bio-methylation of triclosan is in line with a previous study that about 1 % of triclosan is transformed to methyl triclosan under aerobic conditions (Chen *et al.*, 2011). However, another study reported that 42 % of triclosan was transformed into methyl triclosan in the biodegradation assay at 21 ^{o}C (Armstrong *et al.*, 2018). This might be due to their 50 fold lower starting triclosan concentration (about 20 µg/g). The initial concentration of triclosan and methyl triclosan measured in the reactor before spiking with triclosan was 17 µg/L and 0.8 µg/L respectively. Therefore, the elevated concentrations of methyl triclosan (13 µg/L after 168 hours) originated from degradation of the spiked triclosan.

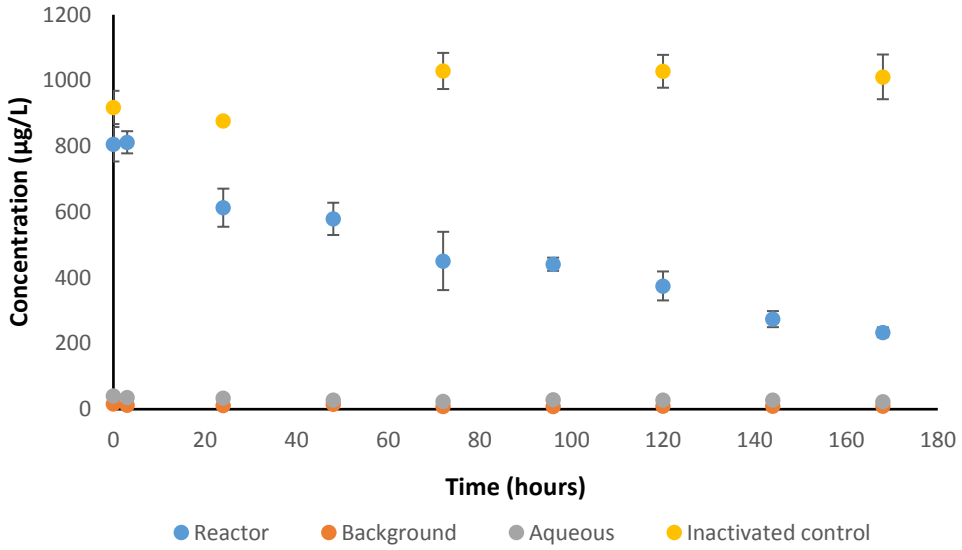


Figure 4-2 Degradation of triclosan over time under aerobic conditions. Error bars represents standard deviation (n = 3). Background and aqueous represents triclosan concentration in the unspiked reactor, and in aqueous phase of spiked reactor respectively

The biodegradation of triclosan is suggested to follow first order kinetics with an estimated half-life ($t_{1/2}$) of 98 hours (Figure 4-4, Table 4-4). However, more complex kinetics was observed for the formation of methyl triclosan in which a fast formation rate was observed until 24 hours before slowing down for the rest of the experiment (Figure 4-4). The rate constant obtained for degradation of triclosan (total phase) were 0.0071 h^{-1}. The degradation rate in this study was lower than those reported by (Chen *et al.*, 2011) (Table 4-4) while the rate of formation of methyl triclosan was faster. This might be due to the difference in experimental parameters such as temperature (17 ^{o}C) and the inoculum concentration (4.0 g/L) used in their studies.

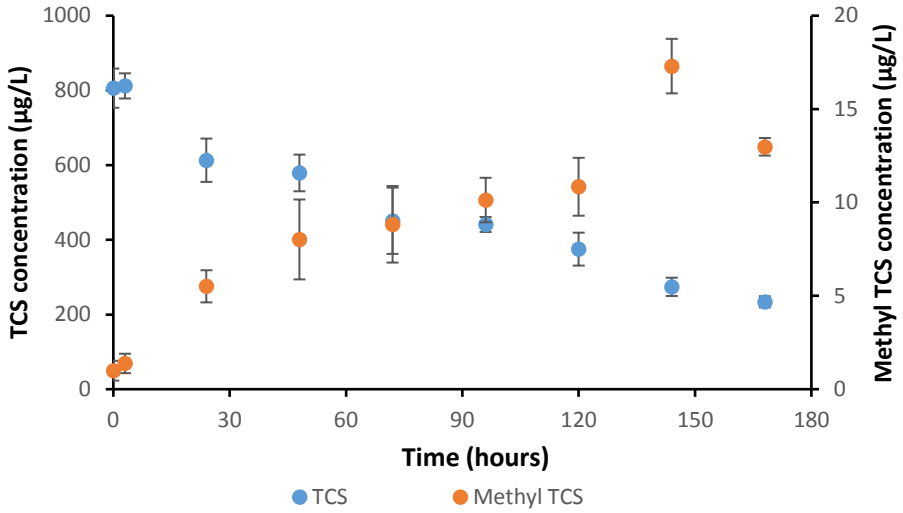


Figure 4-3 Disappearance of triclosan and formation of methyl triclosan under aerobic conditions over time. The error bars represents the standard deviation in triplicate measurements

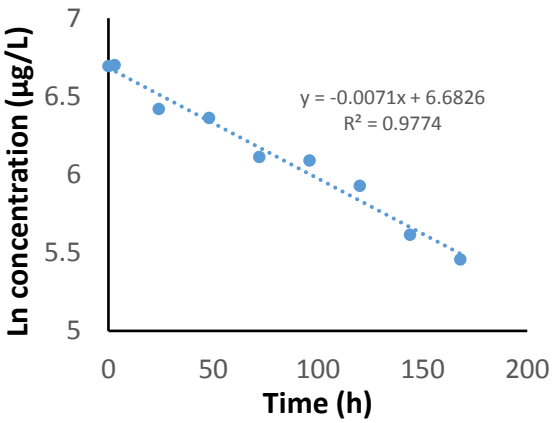


Figure 4-4 Rate plot for degradation of triclosan

Table 4-4 Degradation rate constant of triclosan and formation rate of methyl triclosan under aerobic conditions.

		Disappearance of triclosan				Reference
C_s (µg/L)	C_f (µg/L)	*k* (h^{-1})	S_k	R^2	$t_{1/2}$ (h)	
806	234	0.0071	0.0004	0.9774	98	This work
500	55	0.0095	-	0.9961	73	Chen *et al.*, 2011

C_s and C_f represents starting and final concentration respectively, k represents first order degradation rate constants, S_k represents standard error of the rate constant and $t_{1/2}$ represents the half-life.

4.5.3.2 Biotransformation under anaerobic conditions

After 27 days of monitoring, no reduction of triclosan was observed in both the UASB sludge-inoculated batch test (Figure 4-5) and low temperature biomass-inoculated batch test (Figure 4-6). There was no significant difference between the starting and end concentrations of triclosan in the UASB sludge and low temperature inoculated batch tests (p-value > 0.05, one-way anova). Furthermore, the inactivated control (autoclaved sludge) also stayed at same concentration indicating no reduction of triclosan by abiotic processes. In the low temperature biomass inoculated experiment, triclosan concentrations in both the batch test vessels and inactivated controls were similar, thereby suggesting no degradation occurred. Furthermore, aqueous phase concentrations of triclosan indicated that about 87 – 90 % and 95 – 96 % of triclosan was constantly sorbed to solids in the UASB sludge and low temperature biomass respectively. Methylation of triclosan was not observed in both anaerobic experiments as the concentration of methyl triclosan remained fairly stable from the beginning to the end of the experiment (Figure 4-5, Figure 4-6). Hence, methylation of triclosan only occurs under aerobic conditions.

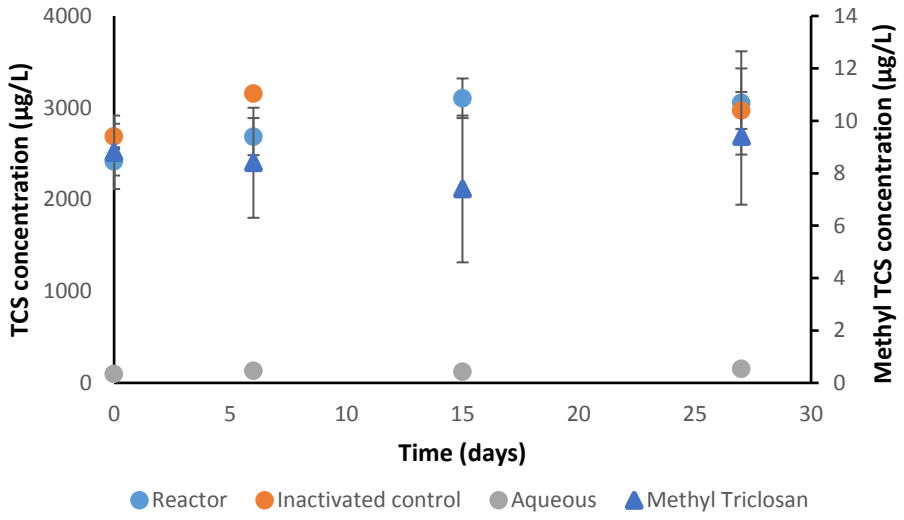


Figure 4-5 Concentration of triclosan (circles) and methyl triclosan (triangles) in the anaerobic batch tests inoculated with UASB sludge. Error bars represents the standard deviation of triplicate measurements

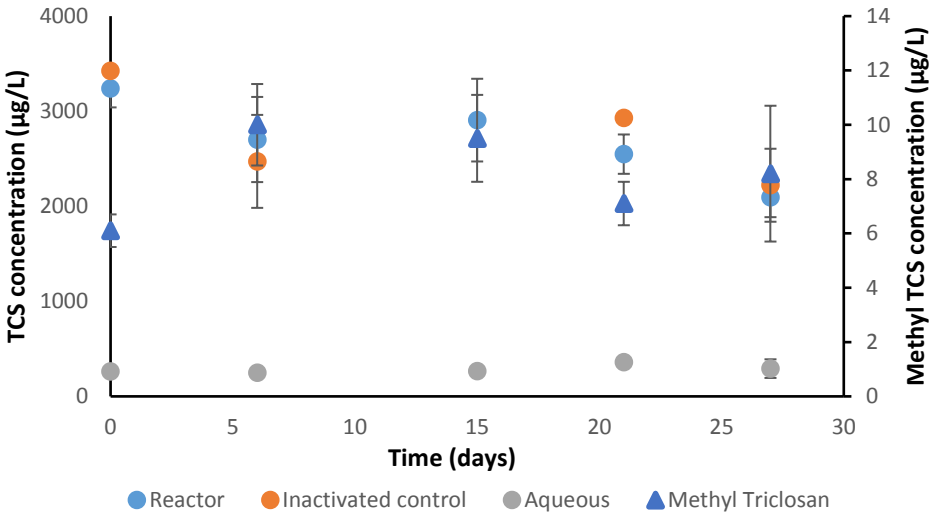


Figure 4-6 Concentration of triclosan (circles) and methyl triclosan (triangles) in the anaerobic batch tests inoculated with low temperature adapted inocula. Error bars represents the standard deviation of triplicate measurements

4.5.4 Degradation of PAHs under aerobic and anaerobic conditions

4.5.4.1 Biotransformation under aerobic conditions

For the 15 PAHs investigated, 10 were degraded aerobically during the 168 h incubation period. The concentrations of six low molecular weight PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene and phenanthrene) (Figure 4-7), and four middle molecular weight PAHs (fluoranthene, pyrene, benz(a)anthracene and chrysene) (Figure 4-8) reduced substantially. There was no reduction in the concentrations of the five high molecular weight PAHs (benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene and benzo(g,h,i)perylene) (Appendix A- Chapter 8). There was no changes in the concentration of all PAHs in inactivated controls except some of the low molecular weight ones (naphthalene, acenaphthylene, acenaphthene and fluorene) (Appendix A- Chapter 8). The reduction of these four low molecular weight PAHs in the control reactor indicates that volatilization played a role in their disappearance from the batch tests. This is as a result of specific properties of those PAHs that favours volatilization, including higher water solubility and lower melting point of these PAHs relative to the heavier ones (Trably *et al.*, 2005). Trably *et al*., also reported abiotic losses of PAHs with less than three aromatic rings under aerobic conditions in a biodegradation experiment carried out using activated sludge inocula (Trably *et al.*, 2005), which is in agreement with the results reported here.

Reduction of low molecular weight (LMW) PAHs ranged from 76 – 95 % in the live batch test vessels (Figure 4-7), and between 12 – 78 % volatilization observed in the autoclaved killed control vessel for all LMW PAHs except phenanthrene and anthracene (Figure 4-9). However, these volatilization percentages were much higher than those predicted (between 0.5 – 2 % for naphthalene, acenaphthylene, acenaphthene and fluorene- see calculation in Appendix C- 8.3) using the Henry's constant of the chemicals at 25 ^{o}C and atmospheric pressure (Shiu and Mackay, 1997; Bamford *et al.*, 1999). This difference might be because the Henry's law volatilization estimates does not consider the effect of aeration in the test vessels on volatilization rates. Especially, as aeration during activated sludge treatment has been reported to intensify the volatilization process (Luo *et al.*, 2014). By comparison, the calculated reduction of middle molecular weight PAHs in the batch tests were lower, ranging from 65 % for chrysene to 77 % for fluoranthene (Figure 4-8). Furthermore, the observed reduction was achieved over 168 hours, and was solely due to biodegradation because no abiotic or non-biological degradation was observed in the inactivated sludge control. These findings are in agreement with reports that degradation of PAHs gets slower with increasing molecular weight under aerobic conditions (Trably *et al.*, 2005).

The half-lives and rate constants for the degradation of LMW PAHs and MMW PAHs were calculated from the data between 24 h to 120 h (except naphthalene, acenaphthylene and acenaphthene between 0 h – 72 h) and 24 h to 168 h respectively. The data at 0 h was omitted in these calculations for all the degraded PAHs due to the observed adaptation phase (where the concentration of the PAHs increased probably due to equilibration and solubilization) in the degradation curve, except in the three LMW PAHs where this did not occur. Regression analysis showed that these omitted data points gave large residual values and its inclusion lead to poorer R^2 values. The disappearance of low and middle molecular weight PAHs was assumed to follow first order kinetics and their half-lives ranged from 17 – 116 hours (Table 4-5), although a scatter around first order kinetics and distinct multiple phases was observed. The half-lives and first order rate constants of LMW PAHs ranged from 11 – 53 hours (naphthalene to anthracene) and 0.0631 h^{-1} – 0.0130 h^{-1} (naphthalene to anthracene) respectively. Since volatilization was observed to contribute the removal of some LMW PAHs, their first order volatilization rates were estimated (Figure 8-16, Section 8.4) and they ranged from 0.0210 h^{-1} for naphthalene, 0.0029 h^{-1} for acenaphthylene and 0.0035 h^{-1} for acenaphthene. In comparison, slower rates and longer half-lives were observed for MMW PAHs with half-lives and first order rate constants ranging from 55 – 116 h (fluoranthene-chrysene) and 0.013 – 0.006 h^{-1} (fluoranthene- chrysene) respectively (Table 4-5). This further suggests that degradation rates of PAHs increases from light to heavy PAH under aerobic conditions, and agrees with findings by several reports in literature (Ghosal *et al.*, 2016). Additionally, the biodegradation rate of individual PAHs are significantly different from each other (p-value < 0.05).

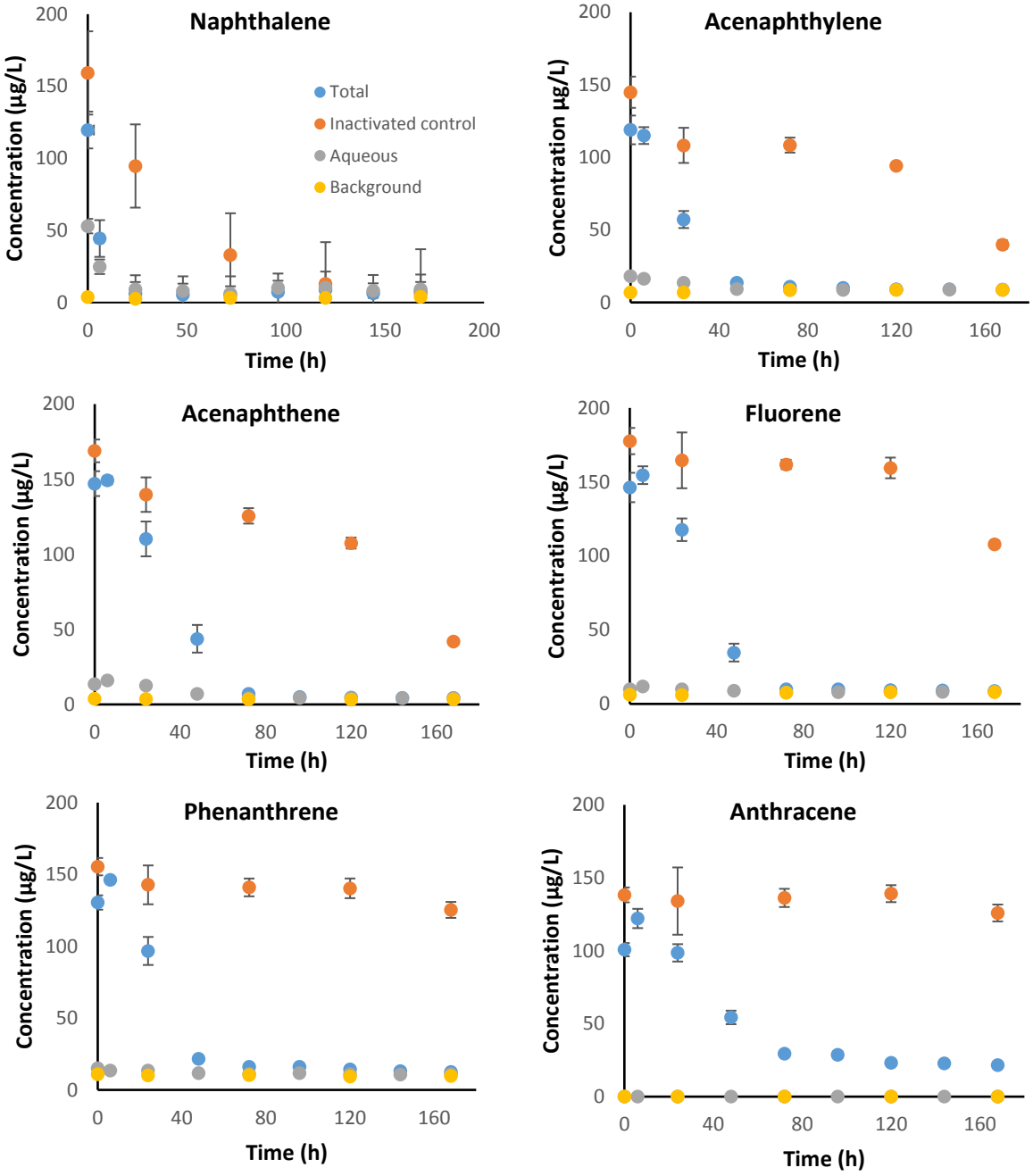


Figure 4-7 Degradation of low molecular weight PAHs over time under aerobic conditions. Error bar represents standard deviation of triplicate measurements

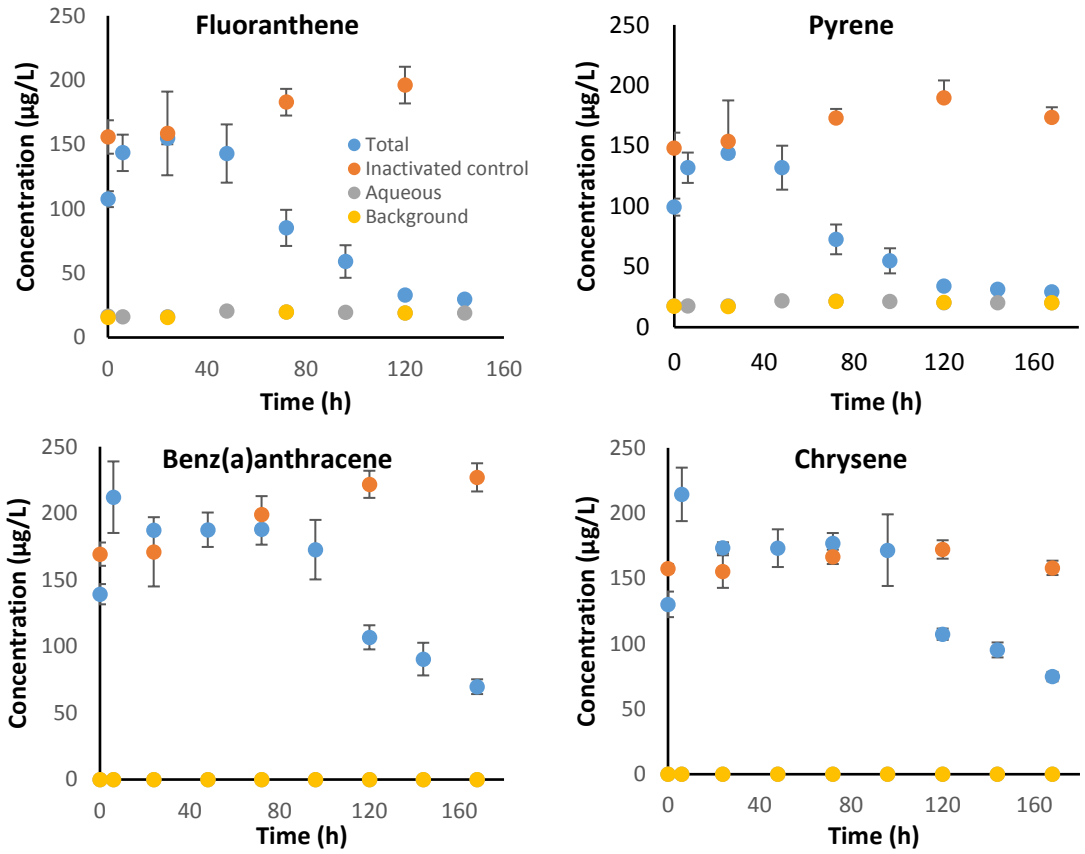


Figure 4-8 Degradation of middle molecular weight PAHs under aerobic conditions. Error bar represents standard deviation of triplicate measurements

Table 4-5 Degradation rate constants (*k*) of PAHs under aerobic conditions with activated sludge incoula

Compound	C_s (µg/L)	C_f (µg/L)	*k* (h^{-1})	S_k	R^2	$t_{1/2}$ (h)
Naphthalene	120	5	0.0631	0.0220	0.8054	11.0
Acenaphthylene	119	11	0.0238	0.0045	0.8477	29.1
Acenaphthene	147	5	0.0291	0.0040	0.8971	23.8
Fluorene	155	9	0.0225	0.0048	0.8153	30.8
Phenanthrene	146	13	0.0172	0.0044	0.7563	40.3
Anthracene	122	23	0.0130	0.0020	0.8900	53.3
Fluoranthene	144	27	0.0126	0.0013	0.9360	55.0
Pyrene	132	29	0.0115	0.0013	0.9332	60.3
Benz(a)anthracene	212	70	0.0067	0.0011	0.8572	103.4
Chrysene	214	75	0.0060	0.0010	0.8539	115.5

C_s and C_f represents starting and final concentration respectively, *k* represents first order degradation rate constants, S_k represents standard error of the rate constant, and $t_{1/2}$ represents the half-life.

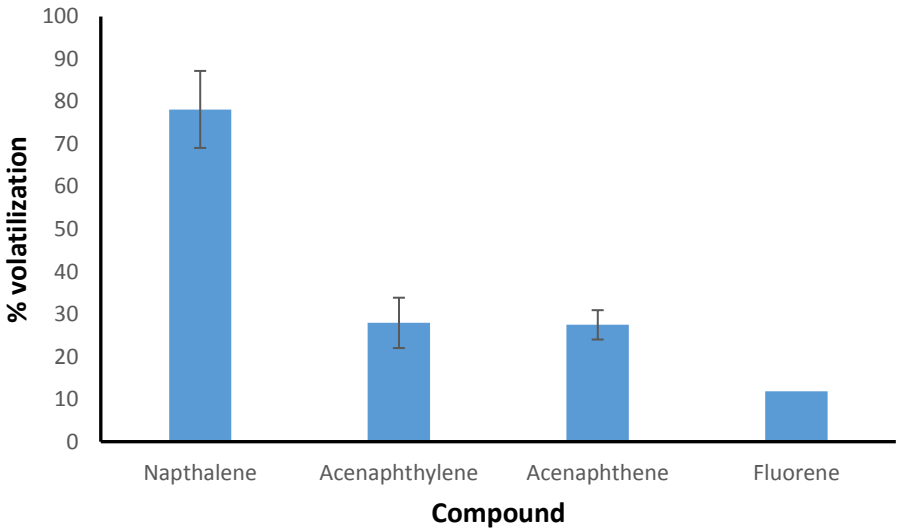


Figure 4-9 Volatilization of low molecular weight PAHs under aerobic condition in the inactivated control incubated at 20 ^oC

4.5.4.2 Biotransformation under anaerobic conditions

None of the 15 investigated PAHs (ranging from low to middle and high molecular weight PAHs) degraded in the anaerobic reactor inoculated with UASB sludge after 27 days of monitoring (Figure 4-10). Furthermore, the concentration of the 15 PAHs in the inhibition and abiotic control remained at the same level throughout the experiment. This indicates that none of the PAHs especially the low molecular weight PAHs were lost due to volatilization or non-biological degradation.

There are no results for the degradation experiment with low temperature sludge inocula since the concentrations of the PAHs across the initial sampling points were close to or below the method detection limits. This may be due adsorption of the PAHs onto the solids in the reactor that could not be extracted with the sampling syringe because of their size.

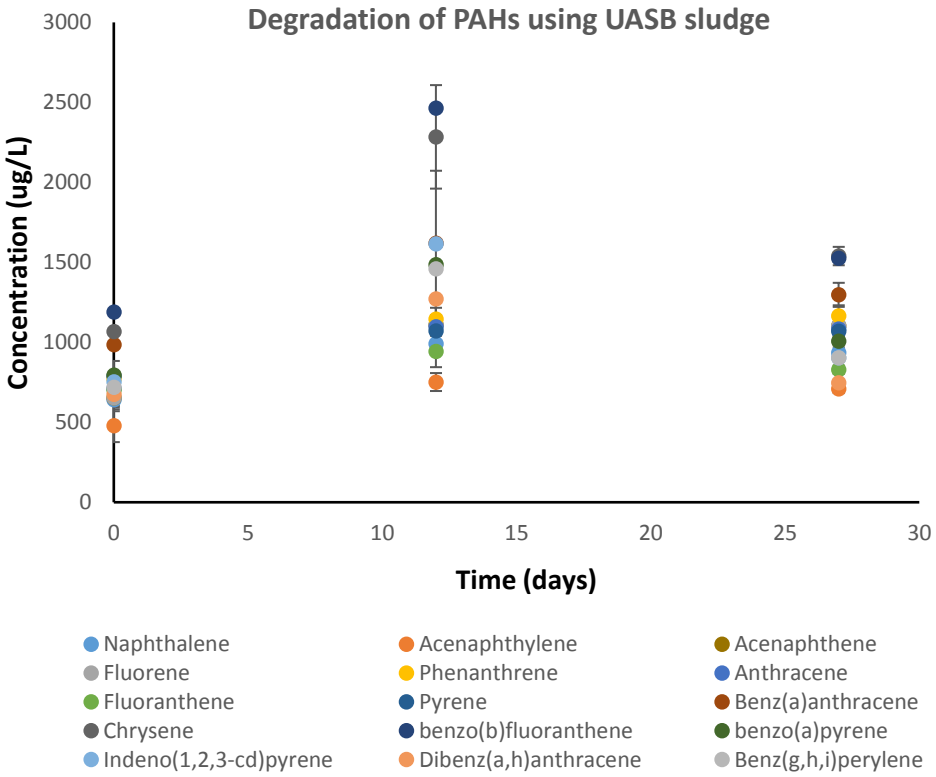


Figure 4-10 Concentration of 15 priority PAHs in the anaerobic reactor inoculated with UASB sludge (initial plot after analysing three sampling plots showed no degradation, so samples between these sampling points were collected but not analysed)

4.5.5 Degradation of estrogens under aerobic and anaerobic conditions

4.5.5.1 Biotransformation under aerobic conditions

The loss of all four estrogens observed in the experiment was solely due to biodegradation as there was no sign of abiotic losses in the inactivated control (Figure 4-11) (this experiment was carried out by (Coello-Garcia, 2018), but the data was analysed and transformed independently). E3 was degraded rapidly within 4 hours and was degraded below the detection limit in 24 hours (Figure 4-11). Also, complete degradation of E2 was observed within 24 hours, however, this was associated with increasing E1 concentration. The measured concentration of E2 reduced by 78 % (from 272 to 60 µg/L) in the first two hours, while that of E1 simultaneously increased by 53 % (from 115 to 175 µg/L) (Figure 4-11, Appendix A- Chapter 8). This observation suggests rapid biotransformation of E2 to E1, and supports findings by several other studies that E1 is a biotransformation product of E2 degradation under aerobic conditions (Dytczak *et al.*, 2008; Racz and Goel, 2010). Over 99.9 % of E1 was removed in 72 hours in the batch tests leaving a residual concentration of 0.1 µg/L (Figure 4-11). Also, synthetic estrogen- EE2 was completely degraded within 72 hours in the batch tests (Figure 4-11). Rapid transformation and high removal rate of E3, E2, E1 and EE2 has been reported to occur under nitrifying conditions with activated sludge and ammonia oxidizing bacteria (Haiyan *et al.*, 2007; Dytczak *et al.*, 2008; Gaulke *et al.*, 2008). Throughout the experiment, E3 was the least adsorbed estrogen as 69 – 96 % was present in the aqueous phase, compared to E1 (about 82 %), EE2 (36 – 48 %) and E2 (5 – 6 %). This might be due to their water solubility and Log K_{ow} (2.47 for E3, 3.13 for E1, 3.67 for EE2 and 4.10 for E2) (Liu *et al.*, 2009). The implication of this is that relatively highly hydrophobic EE2 and E2 possess higher tendency for adsorption when compared to E3 and E1, hence, their removal in treatment plants can be partly due to adsorption (Urase and Kikuta, 2005; Wang *et al.*, 2013a).

The biotransformation of the all four estrogens was assumed to follow first order kinetics with estimated half-lives of 0.8 h, 3.4 h, 5.8 h, and 7.2 h for E3, E2, E1 and EE2 respectively (Table 4-6),), although a scatter around first order kinetics and distinct multiple phases (maybe bi/tri phasic degradation, non-degradation, sorption-desorption) was observed. Furthermore, the rate constants estimated for E3 (0.8941 h^{-1}), E2 (0.2035 h^{-1}), E1 (0.1198 h^{-1}), and EE2 (0.0963 h^{-1}) were noticeably different from each other and indicated that the degradation of E3 was quicker, while that of EE2 was the slowest among the four estrogens (E3 > E2 > E1 > EE2) (Table 4-6). This finding is in agreement with some other studies

(Petrie *et al.*, 2014; Liu *et al.*, 2015), and the recalcitrance and slow degradation of EE2 by activated sludge is also well reported (Combalbert and Hernandez-Raquet, 2010). However, another study reported that E2 was the easiest ($t_{1/2}$ of 0.9 h) to digest while EE2 was the hardest ($t_{1/2}$ of 18.9 d) in their degradation studies with nitrifying activated sludge inoculum and the estrogens as the sole source of carbon (Wang *et al.*, 2013a).

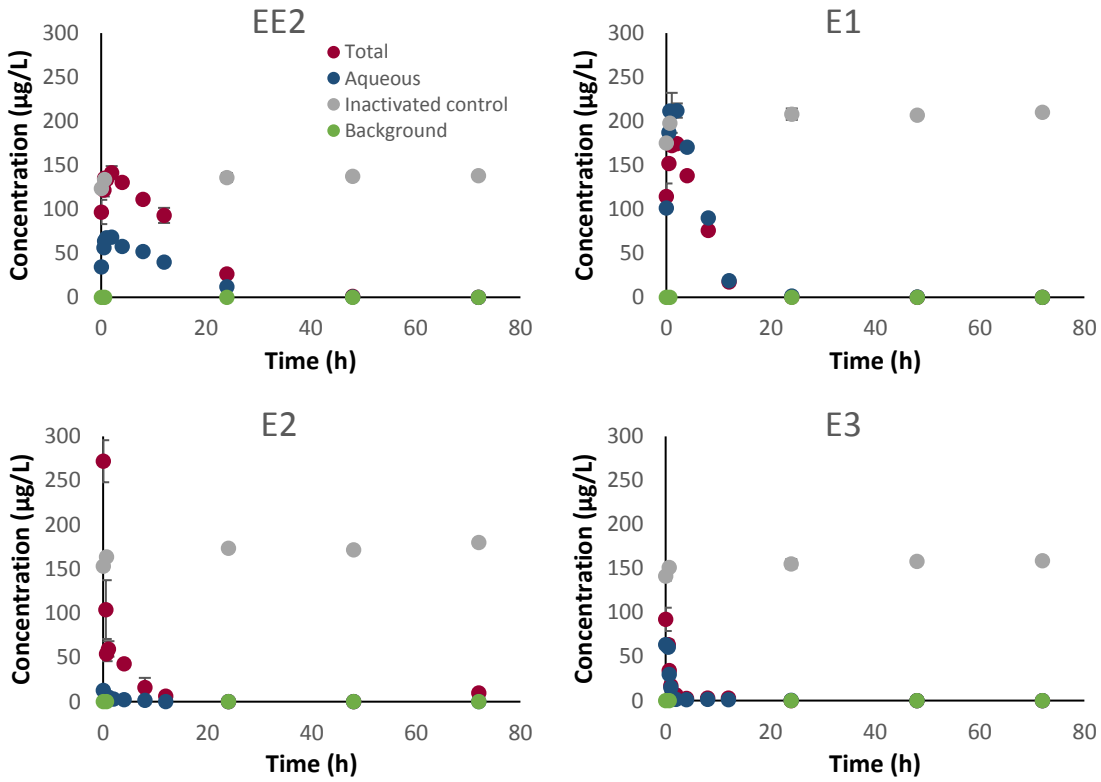


Figure 4-11 Degradation of estrogens over time under aerobic conditions. Error bars represents the standard deviation of replicate measurements

Table 4-6 Degradation rate constants (k) of estrogens under aerobic conditions

Compound	C_s (µg/L)	C_f (µg/L)	k (h^{-1})	S_k	R^2	$t_{1/2}$ (h)
EE2	97	< LOQ	0.0963	0.0088	0.9363	7.2
E1	115	0.1	0.1198	0.1485	0.8578	5.8
E2	272	< LOQ	0.2035	0.0233	0.9273	3.4
E3	92	< LOQ	0.8941	0.0163	0.9006	0.8

C_s and C_f represents starting and final concentration respectively, S_k represents standard error of the rate constant, and $t_{1/2}$ represents the half-life.

4.5.5.2 Biotransformation under anaerobic conditions

Quantification of E2 proved problematic by the chosen analytical method, hence results are only shown for E1, E3 and EE2 from the anaerobic degradation experiment. After 27 days of monitoring, very little or no reduction of E1, E3, and EE2 was observed in both the anaerobic batch tests inoculated with UASB sludge (Figure 4-12) and those inoculated with low temperature inocula (Figure 4-13). The levels of E1, E3 and EE2 also stayed the same for 27 days in the inactivated control, indicating no abiotic losses. The findings of this study, however contrast with some previous studies that reported degradation of estrogens under anaerobic conditions. Andersen (Andersen *et al.*, 2004) and Zhang (Zhang *et al.*, 2015) reported slow degradation of E1, E2 and EE2 with activated sludge under anaerobic conditions. However, another study reported degradation of E1 and E2 but not EE2 under the same conditions (Joss *et al.*, 2004). Czajka & Londry, 2006 also observed no degradation of EE2 but biotransformation of E1 and E2 in their anaerobic degradation study using lake sediment as inocula (Czajka and Londry, 2006). This present work differs from the reported studies, as the anaerobic batch tests were inoculated with UASB sludge or low temperature inocula. The difference in microbial diversity in this UASB or low temperature to the activated sludge inocula might have led to the contrasting observations found in this study. Although, there is limited or no studies on anaerobic E3 degradation in literature to compare, it was interesting to observe resistance of E3 to anaerobic biodegradation.

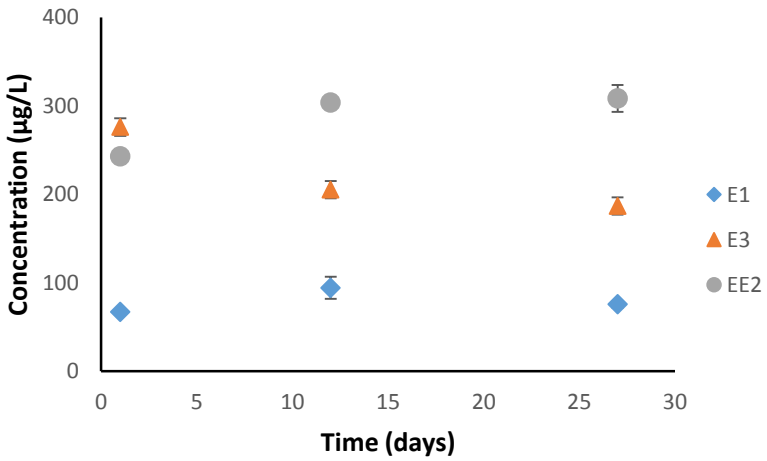


Figure 4-12 Concentration of estrogens in the anaerobic batch tests inoculated with UASB sludge

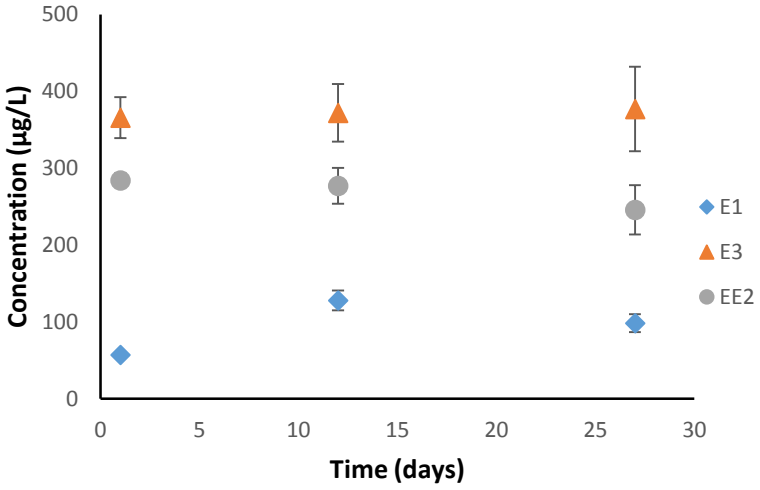


Figure 4-13 Concentration of estrogens in the anaerobic batch tests inoculated with low temperature biomass

4.5.6 **Comparing the degradation rates of the different chemicals under aerobic conditions**

The degradation rates of the different chemicals were compared to understand the effect of chemical structure and functional groups on biodegradability. For comparison, the degradation rates for estrogens at 20 ^{o}C were estimated using the 'effect of temperature relationship' as the experiment was carried out at 15^{o}C. The effect of temperature relationship is based on the fact that temperature influences the metabolic activities of the microbial population and other physical processes such as gas-transfer rates in a biological process (Tchobanoglous *et al.*, 2014).

$$\left(\frac{K_2}{K_1}\right) = \theta^{(T2-T1)}$$

Where K_1 and K_2 = rates at 15 ^{o}C and 20 ^{o}C respectively, T1 and T2 are temperatures 15 ^{o}C and 20^{o}C respectively (in Celsius or Kelvin), and θ is the temperature activity coefficient.

The temperature activity coefficient for most biological systems ranges from 1.02 to 1.10 (Tchobanoglous *et al.*, 2014). Hence, a θ value of 1.08 was assumed to calculate the degradation rates for estrogens at 20 ^{o}C (Table *4-7*).

Table 4-7 Degradation kinetics of estrogens at 15 ^{o}C and 20^{o}C under aerobic conditions

Compound	Rate at 15 ^{o}C	Rate at 20 ^{o}C*	$t_{1/2}$ at 20 ^{o}C
EE2	0.0963	0.1415	4.9
E1	0.1198	0.1760	3.9
E2	0.2035	0.2990	2.3
E3	0.8941	1.3137	0.5

**Rate at 20 ^{o}C estimated assuming Q_{10} value of 2*

Among the different classes of chemicals investigated, estrogens degraded more rapidly with their average reaction rate 16, 52 and 67 times higher than those for low molecular weight (LWM) PAHs, middle molecular weight (MMW) PAHs and triclosan respectively (Table *4-8*). LMW PAHs degraded 4 times faster than triclosan on average. Degradation of two MMW PAHs (fluoranthene and pyrene) was faster than triclosan, while the benz(a)anthracene and chrysene were slower (Table *4-8*). This difference in chemical structure and functional groups among the chemical classes influenced their biodegradation abilities. Under aerobic conditions, biotransformation of aromatics is initiated by hydroxylation (addition of molecular oxygen to the aromatic ring) followed by cleavage of the aromatic ring and then electrophilic substitution (Pitter and Chudoba, 1990). The presence of strong electron

withdrawing substituent groups (such as halogens, nitrogen) decreases the electron density of the aromatic ring and consequently reduces the biodegradation rate in comparison to weaker substituent groups (OH, CH_3) (Pitter and Chudoba, 1990). This explains why the degradation of triclosan was slower than estrogens and some PAHs. Vuono *et al*., reported poor removal of chemicals with strong electron withdrawing functional groups in full scale membrane bioreactors in comparison to other chemicals (Vuono *et al.*, 2016).

Table 4-8 Comparing the degradation rates of different classes of chemicals under aerobic conditions with activated sludge inocula

Compound	Class of chemical	First order rates (h^{-1})	$t_{1/2}$ (h)	Class average rate (h^{-1})
Triclosan	PCP	0.0071	97.6	0.0071
Naphthalene	LMW PAHS	0.0631	17.3	0.0281
Acenaphthylene	LMW PAHS	0.0238	18.5	
Acenaphthene	LMW PAHS	0.0291	20.4	
Fluorene	LMW PAHS	0.0225	19.8	
Phenanthrene	LMW PAHS	0.0172	33.0	
Anthracene	MMW PAHS	0.013	43.3	
Fluoranthene	MMW PAHS	0.0130	53.3	0.0092
Pyrene	MMW PAHS	0.0115	60.3	
Benz(a)anthracene	MMW PAHS	0.0067	103.4	
Chrysene	MMW PAHS	0.0060	115.5	
EE2	Steroidal hormones	0.1362	5.1	0.4645
E1	Steroidal hormones	0.1694	4.1	
E2	Steroidal hormones	0.2878	2.4	
E3	Steroidal hormones	1.2644	0.5	

**PCP represents personal care product; * LMW and MMW represents low molecular weight and middle molecular weight respectively, and are industrial chemicals*

4.5.7 Comparing the degradation rates obtained for these chemicals to those reported in literature

The degradation rates reported in literature varies largely as most of most experiments are conducted under different conditions such as temperature, concentration of the inocula and initial spiked concentration. However, an attempt was made to compare our observed rates with some in literature. For estrogens, the degradation rates and ability for individual estrogens varied largely (Table *4-9*). The decreasing order of degradation in our study; E3 > E2 > E1 > EE2 was different from those reported by (Shi *et al.*, 2004) (E2 > E1 > EE2 > E3), and (Petrie *et al.*, 2014) (E3 = E1 > E2). These contrasting results were also observed in a review on degradation of estrogens by (Liu *et al.*, 2015).

Table 4-9 Comparing degradation rates of estrogens under aerobic conditions in this study to those reported in literature

Compound	Inoculum	Temp. (ºC)	Concentration spiked (µg/L)	Rate	Refs
EE2	Activated sludge (MLSS 3.3 g/L)	15	100	0.0963 h^{-1}	*a*
	Activated sludge (MLSS 2.7 g/L)	30	1000	0.0350 h^{-1}	*b*
E1	Activated sludge (MLSS 3.3 g/L)	15	100	0.1123 h^{-1}	*a*
	Activated sludge (MLSS 2.7 g/L)	30	1000	0.0560 h^{-1}	*b*
	Activated sludge (MLSS 1.0 g/L)	18	0.1	0.3500 h^{-1}	*c*
	Soil amended with cattle manure	10	80	0.1136 d^{-1}	*d*
E2	Activated sludge (MLSS 3.3 g/L)	15	100	0.2050 h^{-1}	*a*
	Activated sludge (MLSS 2.7 g/L)	30	1000	1.3000 h^{-1}	*b*
	Activated sludge (MLSS 1.0 g/L)	18	0.1	0.2700 h^{-1}	*c*
	Soil amended with cattle manure	10	80	0.1100 d^{-1}	*d*
E3	Activated sludge (MLSS 3.3 g/L)	15	100	0.8941 h^{-1}	*a*
	Activated sludge (MLSS 2.7 g/L)	30	1000	0.0300 h^{-1}	*b*
	Activated sludge (MLSS 1.0 g/L)	18	0.1	0.3300 h^{-1}	*c*

References (Refs); *a* = this study *b* = (Shi *et al.*, 2004), *c* = (Petrie *et al.*, 2014), *d* = (Lucas and Jones, 2006)

Estrogens (E1, E2, E3, EE2) have been reported to degrade slower than other ubiquitous steroidal hormones (such as testosterone, progesterone and androstenedione) under aerobic conditions (Esperanza *et al.*, 2007; Yang *et al.*, 2010). Yang *et al*. reported in their degradation study with manure-borne bacteria as inocula that the degradation of testosterone (k = 0.137 h^{-1}) and progesterone (k = 0.120 h^{-1}) was about five time faster than that of E2 (k = 0.025 h^{-1}) under aerobic conditions at 22 ºC (Yang *et al.*, 2010). This implies that WWTPs capable of removing estrogens will easily remove these other steroidal hormones.

Table 4-10 Comparing first order degradation rates of triclosan under aerobic conditions in this study to those reported in literature

Compound	Inoculum	Temp. (ºC)	Concentration spiked	Rate	Refs
Triclosan	Activated sludge (MLSS 3.3 g/L)	20	1000 µg/L	0.0071 h^{-1}	*a*
	Activated sludge (MLSS 4.0 g/L)	17	500 µg/L	0.0095 h^{-1}	*b*
	Activated sludge (MLSS 5.0 g/L)	21	23 µg/g	0.0170 h^{-1}	*c*
	Soil amended with aerobic digestion sludge	23	2000 g/kg	0.0340 d^{-1}	*d*

References (Refs); *a* = this study, *b* = (Chen *et al.*, 2011) , *c* = (Armstrong *et al.*, 2018) *d* = (Wu *et al.*, 2009)

For triclosan, the degradation rates found in this study were close to those reported by (Chen *et al.*, 2011). However, those reported by Armstrong *et al*. (Armstrong *et al.*, 2018) were two times higher; perhaps due to the 50 fold difference in the initial concentration of triclosan (Table *4-10*). Compared to triclocarban (an alternative antimicrobial chemical), triclosan has been reported to be more biodegradable. Armstrong *et al*. reported elimination of triclosan but

resistance of triclocarban to aerobic biodegradation with activated sludge inocula in a recent study (Armstrong *et al.*, 2018). Lower first-order degradation rates were also reported for triclocarban in agricultural soils under aerobic conditions compared to triclosan (Cha and Cupples, 2010). The degradation of estrogens and triclosan in a different environmental compartment (soil) is very slow when compared with activated sludge (Table *4-9*, Table *4-10*) possibly because of the differences in the bacterial population of the two inocula. Information on degradation rates of PAHs with both activated sludge and soil is scarce in the literature therefore, they were not included in this comparative section.

4.5.8 **Predicting effluent quality and associated risks using obtained degradation rate constants**

The obtained biodegradation rates were used to predict effluent concentrations and assess the likelihood of risk when these chemicals are discharged into water bodies. In a completely stirred tank reactor (CSTR), effluent concentration is given by the equation below (Levenspiel, 1999);

$$Predicted\ effluent\ concentration = \frac{Influent\ concentration}{1 + k\ x\ HRT}$$

Where k = biodegradation constant (h^{-1}), HRT = hydraulic retention time of the activated sludge plant sampled (10.58 h), and concentrations are in ng/L.

The following assumptions were made in these estimations;

1. 85 % of the influent concentration was assumed be remove by sorption to solids during primary and secondary treatment. (Lozano *et al.*, 2013) reported that 88% of triclosan was removed via adsorption during primary and secondary treatment of an activated sludge plant. Since, the Log K_{ow} of triclosan (4.8) is similar to those of anthracene (4.6) and fluoranthene (5.2) (see Section 3.1.1), removal rate via sorption might be similar.
2. Removal due to flow was not considered

Table 4-11 Predicted effluent concentration of the chemicals after activated sludge aerobic treatment

Compound	Measured inf conc (ng/L)	Conc after sorption (ng/L)	Rate (h^{-1})	Predicted eff conc (ng/L)	Measured eff conc (ng/L)	EQS standard (ng/L)
Triclosan	13117	1967.6	0.0071	1830.1	1443	100
Naphthalene	689.4	103.4	0.0400	72.7	211.1	130,000
Acenaphthylene	191.5	28.7	0.0374	20.6	63.3	-
Acenaphthene	182.9	27.4	0.0340	20.2	35.1	-
Fluorene	277.8	41.7	0.0350	30.4	47.8	-
Phenanthrene	633.7	95.1	0.0210	77.8	87.7	-
Anthracene	421.1	63.2	0.0160	54.0	95.3	100
Flouranthene	743.1	111.5	0.0130	98.0	101.4	120
Pyrene	691.2	103.7	0.0115	92.4	99.8	-
Benz(a)anthracene	754.4	113.2	0.0067	105.7	181.3	-
Chrysene	556.2	83.4	0.0060	78.4	129.5	-

- *The five high molecular PAHs were not included because no biodegradation rates were obtained for them*
- *The concentration of estrogens were not determined in this plant, hence they were not included.*
- *Conc represents concentration; inf and eff represents influent and final effluent respectively.*
- *Concentrations of the chemicals used was previously reported in Chapter 3*

The predicted effluent concentration for triclosan (1830 ng/L), anthracene (54 ng/L) and flouranthene (98 ng/L) was close to the measured values (Table *4-11*). The predicted effluent concentration of triclosan was well above the EQS standard while those of naphthalene, anthracene and flouranthene were below (Table *4-11*). Hence, effluent from this treatment plant would pose a risk to aquatic organisms when discharged into receiving waters. To avert this, an HRT of 2630 h is required to reduce the concentration of triclosan below the EQS value (100 ng/L in freshwater), which is non-practical. However, a river dilution factor of at least 18 times might ensure compliance to the EQS standard.

4.5.9 Microbial diversity and enriched bacterial taxa associated with PAH degradation

4.5.9.1 Microbial diversity and species enrichment

Relative abundances of different bacterial families and genera increased or decreased after incubation with, and the biodegradation of PAHs (Figure 4-14). This is unsurprising as some microorganisms reportedly have the capacity to grow on PAHs containing between two to four rings (Jones *et al.*, 2014). This might explain why degradation of only low-middle molecular weight PAHs but not high molecular weight PAHs were observed in this study. Some of the genera whose relative abundance increased significantly ($p < 0.05$, G's test) at the end of the biodegradation assay included *Methyloversatilis, Dechloromonas, Dokdonella, Mycobacterium, Zymomonas, Acidiphilium and Turicibacter* (Figure 4-14).

Among these enriched genera, *Mycobacterium* has been extensively associated with degradation of PAHs in several studies (Peng *et al.*, 2008; Ghosal *et al.*, 2016). (Dean‐Ross *et al.*, 2002) and (Churchill *et al.*, 2008) reported simultaneous degradation of pyrene, fluoranthene and phenanthrene by different *Mycobacterium sp* isolated from contaminated sediments. *Mycobacterium vanbaalenii* PYR-1 has also been reported to simultaneously degrade low to middle molecular weight PAHs including naphthalene, anthracene, henanthrene, fluorantene and pyrene through oxygenase-mediated metabolism (Moody *et al.*, 2001; Kim *et al.*, 2005). Furthermore, a high abundance of *Mycobacterium* detected in microbial samples from a beach polluted with oil spill was associated with degradation of PAHs (Alonso-Gutiérrez *et al.*, 2009). Another enriched genera *Acidiphilium* was reported as a PAH degrading bacteria under strongly acidic conditions (Stapleton *et al.*, 1998). However, its enrichment in this study suggests that they might also have used the PAHs as growth substrate under close to neutral (5 – 6.5) pH conditions.

On the other hand, there was also a significant reduction ($p < 0.05$) in some of the genera after the biodegradation assay (Figure 4-14). Some of those genera of note are *Sphingobium* and *Novosphingobium* that have been reported to possess great catabolic versatility and capability to degrade a wide range of xenobiotic compounds including PAHs (Peng *et al.*, 2008; Vila *et al.*, 2015). These PAH degraders along with other genera that were reduced might have unsuccessfully competed for available nutrients and substrate from the activated sludge inocula.

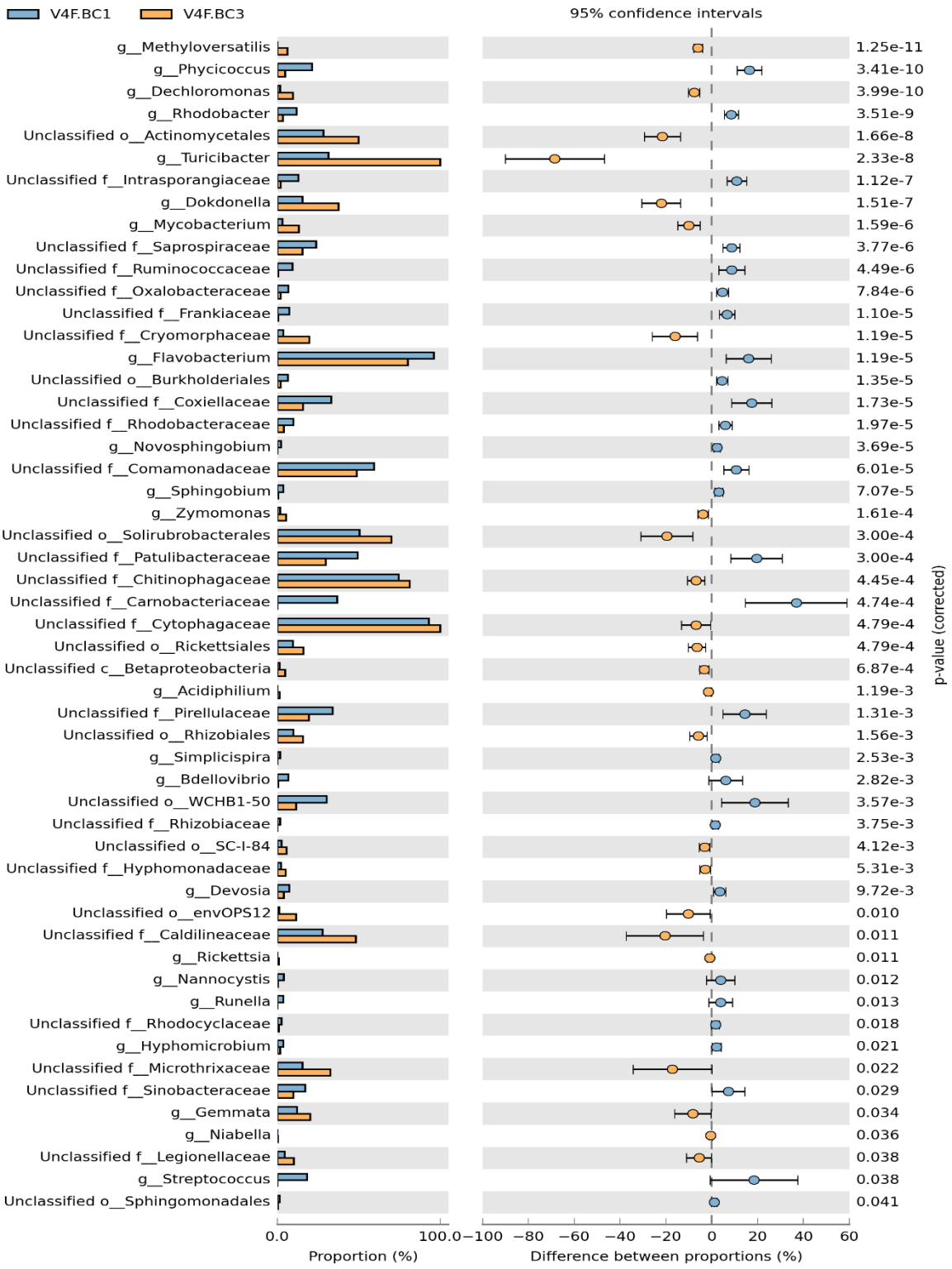


Figure 4-14 Extended error bar plot comparing the relative abundance of metagenomic profile for the PAHs biodegradation test using activated sludge inocula (Genus profile level to Class parent level). Significantly different genera (P-value < 0.05, G-test + Fisher's) between the start and end of the experiment was presented

Additionally, a ranking of the OTUs at genus level based on their relative abundances before and after the PAH degradation experiments shows that there was an increase in the relative abundance of certain genera over others. This resulted in an increase in their rank abundance and vice versa, thereby showing a significantly different rank-abundance pattern (Figure *4-15*). Some known PAHs degraders; *Mycobacterium* and *Acidiphilium* were significantly enriched, and their resultant rank improved from 67 to 48, and 134 to 82 respectively. However, the rank of some other known PAHs degraders *Novosphingobium* and *Sphingobium* that were significantly reduced increased from 50 to 122, and 68 to 110 respectively.

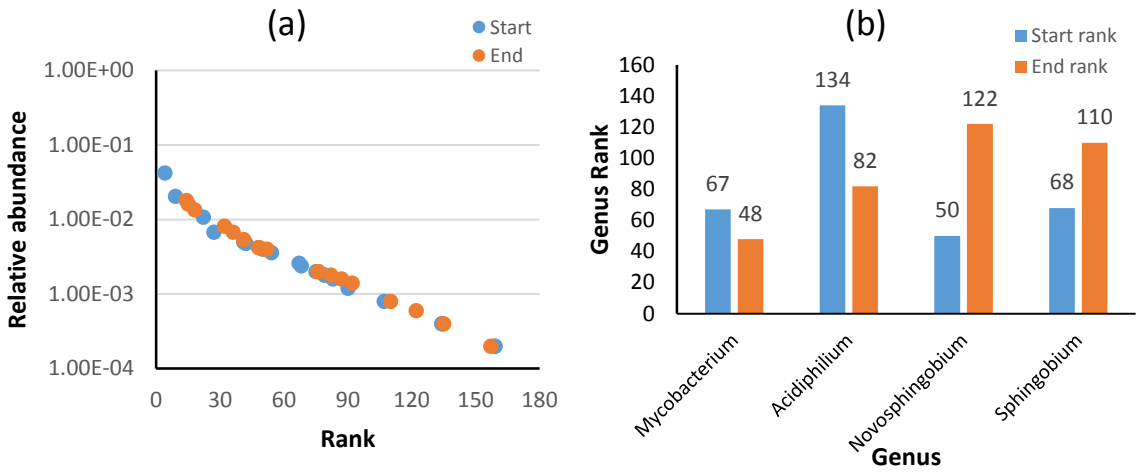


Figure 4-15 (a): Relative abundance of genera that significantly changed over the duration of the PAHs degradation assay in rank order. (b): The rank order of some known PAH degraders at the start and end of the assay

4.5.9.2 Distribution of rare and abundant taxa in microbial community

Figure *4-16* shows the distribution of different genera and their abundance at the start and end of the PAH degradation experiment. The calculated frequencies of genera were based on even depth of 5000 sequences per sample to accommodate samples with low reads (5000 sequences was the minimum). The genera above the x = y line were enriched after the degradation assay while those below were impoverished.

Furthermore, the role of rare and abundant taxa in the inocula on degradation of the PAHs were studied as described by (Vuono *et al.*, 2016). 1 % abundance was set as the upper threshold of rarity in this study such that genera at < 1 % abundance and ≥ 1 were classified as rare and abundant taxa respectively. In this study, most of the known PAH degraders such as *Mycobacterium* and *Acidiphilium* are rare taxa as their relative percentage abundance was < 1 % (Figure *4-16*). Thereby suggesting that rare taxa plays an important roles in degradation of chemicals in activated sludge systems.

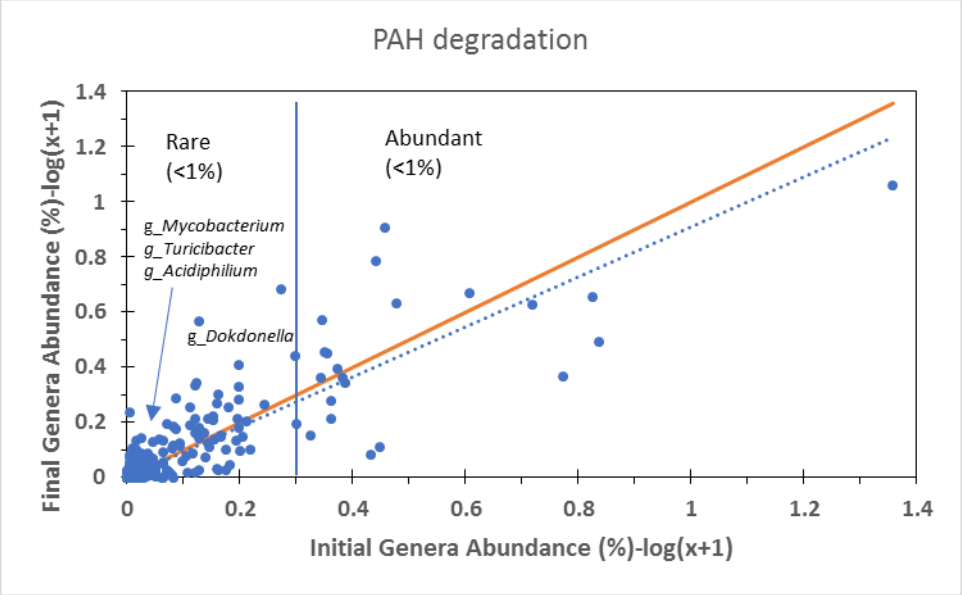


Figure 4-16 Relationship between final and initial percentage abundances of the genera in the PAHs biodegradation experiment under aerobic conditions with activated sludge inocula. The orange line is x = y line, the dotted blue line represents the fitted trend line for the regression analysis and the solid vertical blue line separates the rare and abundant taxa

4.5.9.3 Inter-relationship between the micro bacterial communities

There was a shift in microbial community after degradation of PAHs as the start (R_St1 and R_St2) and end (R_End1 and R_End2) communities clustered separately (Figure 4-17). Furthermore, the microbial community in the control batch tests (not spiked with PAHs) clearly differed from the start community. This suggests that the microbial community shifted, which may be due to competition of available nutrients over time and subsequent enrichment or reduction of competing genera. Duplicate samples (R_St1 and R_St2) at the beginning of the experiment clustered together, thereby indicating very similar microbial communities. However, duplicate samples (R_End1 and R_End2) after degradation of PAHs were clustered over a wider area indicating a larger community spread even in duplicates.

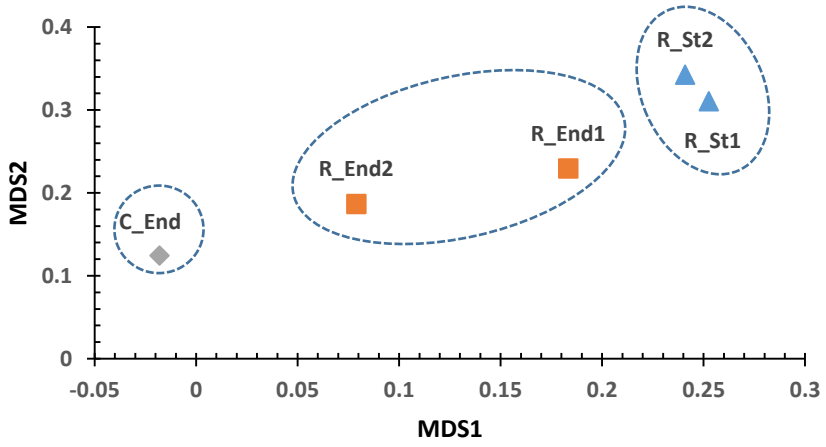


Figure 4-17 Multidimensional scale plot comparing the bacterial communities at genus level present in the aerobic batch tests before and after PAHs degradation. Clusters for duplicate samples and various stages are circled separately. C refers to the control (not spiked with PAHs) and R refers to the batch tests spiked with PAHs. St and End represents beginning and end of the experiment. 1 and 2 are replicates.

4.6 Conclusion

In this study, degradation of different classes of chemicals (triclosan, 15 PAHs and 4 estrogens) from a wide range of domestic and industrial applications were studied with laboratory-scale batch experiments under aerobic and anaerobic conditions.

All the investigated chemicals except high molecular weight PAHs degraded under aerobic conditions. The first order degradation kinetics was in the following decreasing order for the chemicals E3 > E2 > E1 > EE2 > triclosan > naphthalene > acenaphthylene > acenaphthene > fluorene > phenanthrene > anthracene > flouranthene > pyrene > benz(a)anthracene > chrysene, showing that estrogens (steroidal hormones) were the least persistent amongst the different classes of chemicals while middle molecular weight PAHs were the most persistent. Furthermore, the degradation of similar chemicals within the same class such as testosterone and progesterone (steroidal hormones) will be faster than estrogens while triclocarban (personal care product) will degrade much slower than triclosan in activated sludge systems. Additionally, the effluent concentration predicted with the obtained first order degradation rates showed that the concentration of triclosan was above its EQS value and would pose risk to aquatic organisms when discharged into receiving waters- unless the river dilutes it by at least 18 times. However, the synergistic effect of the cocktail of different classes of compounds on aquatic organisms remains unknown

Triclosan degradation resulted in the concomitant formation of methyl triclosan, a more persistent and lipophilic metabolite. The molecular weight of individual PAHs influenced their removal in these system as their degradation was observed to reduce with increasing molecular weight. E1 was identified as a major metabolite of E2 degradation, as over 58 % of the degraded E2 was converted to E1. Volatilization was also observed to contribute majorly to the removal low molecular weight PAHs in this experiment. Furthermore, in real wastewater treatment systems, sorption will contribute to the initial removal of triclosan, middle molecular weight PAHs, E1, E2, and EE2 as observed in the biodegradation test. Additionally, amongst the degraded chemicals only the estrogens were almost completely degraded which means they might be degraded within the operational time frame of an activated sludge plant.

A significant change in the metagenomics profile of the inocula spiked with PAHs at the end of the biodegradation assay was observed after taxanomic analysis of the bacterial communities. Furthermore, known PAHs degrading genera- *Mycobacterium* and *Acidiphilium* were enriched after significantly after PAH degradation and their rank abundance increased

resultantly when compared to the un-spiked inocula. Additionally, a shift in the microbial community was observed at the end of the degradation assay following fortification with PAHs.

Under the two anaerobic conditions (UASB and low temperature biomass inoculated reactors) studied, degradation of any of the chemicals was not observed after 28 days. Therefore, in an operating full scale UASB treatment plant, removal of these chemicals will be most likely attributable to sorption unto sludge, not biodegradation.

Chapter 5: Investigating the fate of micropollutants in Brazil WWTPs

Chapter 5 Investigating the fate of different classes of micropollutants in aerobic, anaerobic and facultative WWTPs in Brazil

5.1 Introduction

The occurrence of steroidal hormones (such as estrogens- E1, E2, E3 and EE2), personal care products (such as triclosan) and industrial chemicals (such as polyaromatic hydrocarbons PAHs; and polybrominated diphenyl ethers PBDEs) in raw wastewater and treated effluents around the world has been reported in ng/L or µg/L levels (Wang *et al.*, 2013c; Luo *et al.*, 2014). The adverse effects of estrogens (Combalbert and Hernandez-Raquet, 2010), triclosan (Singer *et al.*, 2002), PAHs (Jones *et al.*, 2012) and PBDEs (Cristale *et al.*, 2013) on aquatic organisms have been reported, thereby intensifying research efforts over the last two decades on better technologies to remove such pollutants to harmless levels before their release into water bodies. Despite the many reports on the occurrence of these chemicals around the world (see literature review), there are limited studies in Brazilian wastewater and receiving waters. This is of urgent concern as the risk of exposure is as great or even greater since manufacturing in low-middle income countries (LMIC) is as high as in high income countries (HIC) and there are less regulatory and wastewater treatment barriers to protect wildlife and humans from exposure (Weiss *et al.*, 2016). There are only few studies on the occurrence and removal of estrogens in Brazilian WWTPs (Froehner *et al.*, 2010; Pessoa *et al.*, 2014), and one report on the concentration levels of triclosan in surface water in Brazil (Santos *et al.*, 2016). To the best of the author's knowledge, there has been no report on the levels of PAHs and PBDEs in Brazilian WWTPs and water bodies. The relative shortage of studies on micropollutants in Brazil and other LMIC might be due to the difficulties and high costs associated with chemical analysis – i.e. know-how and affordability (Pessoa *et al.*, 2014). Therefore, one of the objectives of this study was to investigate the occurrence and removal of different chemical classes four estrogens (natural hormones and a pharmaceutical), triclosan (personal care products), fifteen PAHs (industrial chemical), and eight PBDE congeners (flame retardant in household products) in different types of real Brazilian wastewater treatment plants.

As far as wastewater treatment technologies are concerned, activated sludge is the worlds' most widely used secondary treatment process, especially in HICs and have been shown to be one of the better technologies for the oxidative removal of micropollutants. Hence, most of the worldwide studies on micropollutants removal refer to this system (Luo *et al.*, 2014). However, these systems are energy intensive- they account for up to 1 % of UK electricity use

(Gardner *et al.*, 2012), and yet are ineffective in reducing this emerging contaminants to environmentally safe levels. Whereas, in LMICs like Brazil, wastewater treatment technologies with relatively lower energy requirements and operational costs such as waste stabilization ponds (WSPs) and up-flow sludge blanket reactors (UASBs) are often used. As the water industry needs to move away from unsustainable energy-costly treatment systems to more sustainable low energy systems, studying the Brazilian systems could benchmark such technologies against each other since they are likely to be part of the future wastewater treatment technologies in temperate climates too. Froehner *et al*. (Froehner *et al.*, 2010) and Pessoa et al. (Pessoa *et al.*, 2014) have previously investigated the removal of estrogens in three Brazilian WWTPs utilizing different technologies – activated sludge, UASB and WSP. However, in their work, they only reported the total removal achieved by the plant without considering the individual contribution of the different treatment stages; and their reported estrogen concentrations are not realistic (see Section 5.3.2.3). To our knowledge there have been no similar studies for other chemical classes and specifically for triclosan (PCP), PAHs (industrial chemical) and PBDEs (flame retardant in household products). Therefore, the second objective of this study was to investigate the behaviour and removal of four classes of micropollutants with different physio-chemical properties (estrogens, triclosan, PAHs and PBDEs) in wastewater treatment plants utilizing different technologies (conventional activated sludge and low energy systems- UASB and WSP); with emphasis on removal achieved after primary and secondary treatment individually. Comparing the effectiveness of these low- and high-energy technologies in removing different groups of pollutants will provide insights on better removal systems and potentially help to implement appropriate technologies for specific contaminants.

Biodegradation and sorption are the main mechanisms of xenobiotic removal in wastewater treatment plants, with volatilization only playing a minor role (Verlicchi *et al.*, 2012). During biological wastewater treatment, micropollutants are either sorbed onto solids or biodegraded-complete mineralization or incomplete degradation and formation of biotransformation products (Luo *et al.*, 2014). Photodegradation has also been reported as a removal mechanism in waste stabilization ponds (Gomez *et al.*, 2007). Therefore, it is important to understand the removal mechanisms of these emerging contaminants in WWTPs using different forms of biological treatment. There have been several studies on the biodegradation of estrogens, triclosan, PAHs and PBDEs under aerobic conditions to understand their removal in aerobic systems such as activated sludge (Alvarino *et al.*, 2014). However, there are limited studies on their fate under anaerobic conditions (as applied in UASB systems) except for estrogens.

Also, there has been no previous attempt to understand the degradation of these group of compounds in WSPs. Hence, the final objective of this studies was to investigate the biodegradability of the selected group of micropollutants under different redox conditions (aerobic or anaerobic) employed by the surveyed activated sludge, UASB and WSP based WWTPs in Brazil. The photodegradation potential of the micropollutants was also investigated in the WSP system. This study will provide insights on the key removal mechanisms of these micropollutants in the different wastewater treatment systems. Additionally, knowing the extent of degradation (degradation rates) of these chemicals in aerobic and anaerobic systems is required to understand the limits of engineering biological systems (e.g. required HRT) for the removal of such chemicals.

5.2 Experimental methods

5.2.1 Materials

Details on analytical standards, solvents, filters, solid phase extraction cartridges for all the compounds are given in Chapter 3 and Chapter 4. HPLC grade methanol, acetone, MTBE, iso-propanol were obtained from Casa Lab or Biosan (Brazil). PCB 209 (10 µg/ml in heptane) and 4PC-BDE-208 (50 µg/ml in toluene), which were used as surrogate standards for PBDE analysis were purchased from Sigma Aldrich (UK), and Wellington Laboratories (via Greyhound Chromatography UK) respectively with purities higher than 98%.

5.2.2 Wastewater treatment plants and sampling

Three WWTPs in and around Belo Horizonte (Brazil) with different treatment processes treating domestic wastewater were selected in this study. Belo Horizonte is the third largest metropolitan area in Brazil with a population of over five million people covering an area of 9,400 km^2 (Travel-Guide, 2018). It is situated in the southeast region of Brazil and its main industries are vehicles, food products, textile, mineral processing and chemicals (Gray, 2009). WWTP A is the biggest wastewater treatment facility in the city and consists of preliminary treatment, primary treatment, and secondary treatment (activated sludge). It has an installed capacity to treat 290,000 m^3/day (operational flow of 200,000 m3/day) of wastewater and serves about 2.3 million people (operational population equivalent (p.e.) of 1.6 million people). The plant uses activated sludge for secondary treatment with a hydraulic retention time (HRT) and solid retention time (SRT) of 4.2 hours and 12 days respectively. WWTP B also consists of preliminary treatment and secondary treatment. It is capable of treating wastewater at 155,000 m^3/day (operational flow of 133,000 m^3/day) with a population equivalent of 1.2 million people (operational p.e. of 1.1 million people). The secondary

treatment process employed here is anaerobic (UASB) with an HRT and SRT of 7.7 hours and 40 days (see calculation in Appendix F- 8.7) respectively. The wastewater then goes to a trickling filter system with a percolating time of 20 – 25 minutes followed by a sedimentation tank with an HRT of 6.1 hours to complete the secondary treatment process. WWTP C is the smallest of the selected treatment plants with an operational capacity to treat 1,600 m^3/day of wastewater and serves about 15,000 people (installed p.e. of 30,000 people). This WWTP is a waste stabilization pond (WSP) system that consists of an anaerobic pond followed by a facultative pond with an HRT of 2 days and 20 days respectively.

These three WWTPs were selected for two reasons. Firstly, they serve large populations that are therefore likely contain the compounds of interest. Secondly, they represent the three main treatment processes used for wastewater treatment in Brazil - from the expensive energy-intensive activated sludge system to the cheaper less energy-intensive UASB and WSP systems. Sampling was carried out in summer season in Brazil (December 2016 – February 2017). As summer in Brazil is usually wet, sampling was usually done after 2 days of dry weather when possible. However, this was not possible during the survey of the WSP (WWTP C). The flow during sampling (74 L/s) was higher than the normal flow (20 – 25 L/s), hence, concentrations were expected to be three times lower than normal. Water samples were collected from various points in the treatment system in all three WWTPs (Figure 5-1). Influent and effluent samples were collected after preliminary and secondary treatment processes respectively for all WWTPs. Primary effluents were sampled after the primary sedimentation tank, UASB reactors and the anaerobic pond for WWTP A, WWTP B, and WWTP C respectively. Emphasis was placed on removal of micropollutants in the biological processes.

In WWTP B, the influent was distributed into twenty-one UASB reactors operated in parallel. Therefore, sludge samples were collected from three of the reactors (one at the beginning, middle and end of the train) and mixed together for a better representation of the plant. Furthermore, samples were initially collected from the same reactor height (3 sampling points present at 0.3 m, 0.8 m and 1.3 m) to obtain comparable solids concentration from each reactor. Samples were eventually collected at 0.8 m, as samples at 0.3 m and 1.3 m were too concentrated or too diluted respectively. Grab liquid and solid samples were collected in cleaned and disinfected (with 1% Virkron for 24 hours, followed by several rinse cycles with distilled water) 5 L high-density polyethylene (HDPE) containers; analysis of the empty rinsed containers showed no contamination with any of the target compounds. Samples were stored at 4 ^{o}C upon arrival to the laboratory and were used within 48 hours.

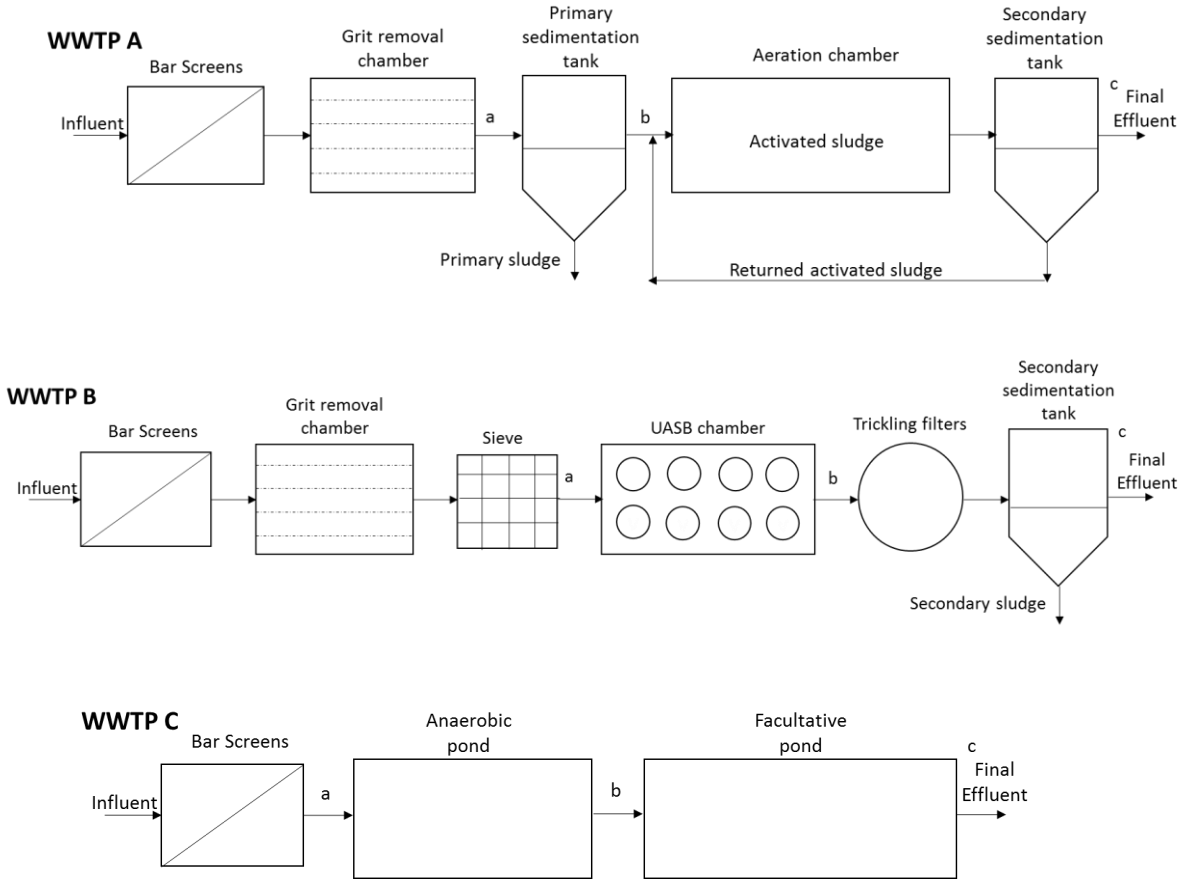


Figure 5-1 Schematic diagram of the three treatment process streams studied at Brazilian WWTPs. The sampling points for influent, primary effluent and secondary effluent collection is indicated by a, b and c respectively

5.2.3 **Wet chemistry analysis for the WWTPs**

Suspended solids (SS) and chemical oxygen demand (COD) were measured in the three WWTPs, which were important in assessing the general performance of the WWTPs. The total suspended solids (TSS) and volatile suspended solids (VSS) content of the liquid and solid samples were measured according to the Standard Method 2540B, while COD was measured according to Standard Method 5220D (Clesceri *et al.*, 2005).

5.2.4 **Analysing the micropollutants in wastewater**

The concentration of triclosan, 15 priority PAHs, 4 estrogens and 8 PBDE congeners were determined in the raw influent, primary effluent and secondary effluent of the three investigated WWTPs. Triclosan and PAHs were quantified according to methods described in Section 3.1.2, estrogens with methods employed by (Coello-Garcia, 2018), and PBDE according to methods described in Section 3.2.2. Sample extraction was carried out by solid phase extraction (SPE) and analysed by GC-MS for triclosan and PAHs, LC-MS/MS for estrogens and GC-ECD for PBDEs. To extract these compounds in the aqueous phase of the

samples, 500 mL of filtered (Sartorius MGB filters, 0.7mm thick, 1.0 µm particle retention) wastewater was processed for triclosan and PAHs, 150 – 250 mL for estrogens, and 50 – 100 ml for BDEs to measure their concentrations in the aqueous phase only. For all these compounds, except estrogens, combined aqueous and particulate matter (PM) phases were analysed by using 50 mL of unfiltered influent and 200 mL of unfiltered effluent percolated through the SPE cartridge for triclosan and PAHs (Sánchez-Avila *et al.*, 2009). 20 mL and 50 mL of unfiltered influent and effluent respectively were used for extraction of total PBDEs (i.e. particulate and aqueous phases).

5.2.5 **Experimental design of biodegradation assay**

Aerobic biodegradation experiments for triclosan, PAHs and estrogens were carried out as described in Section 4.2.3 with few modifications. Briefly, batch tests were placed in an incubator equipped with a shaker (Marconi MA420), which was operated at 140 rpm for homogenous mixing. The experiment was carried out at room temperature (28 – 32 °C) as the incubator was not a cooling incubator. However, the temperature for the duration of the experiment was recorded with a temperature logger (Lascar Electronics, EasyLog EL-USB-2-LCD). Dissolved oxygen (DO) and pH were monitored throughout the experiment to ensure that they were not limiting or excessive, respectively; they ranged from 0.25 – 0.45 mg/L DO and pH of 6.6 – 7.3 respectively. This WWTP operates at a low dissolved oxygen concentration (0.2 – 0.3 mg/L) as they are not mandated to remove nitrogen, thereby saving on energy costs. The batch tests were sampled at multiple points (0, 6, 24, 48, 72, 96, 120, 144, and 168 hours for triclosan and PAHs; 0, 0.3, 0.7, 1, 2, 4, 8, 24, 48 and 72 hours for estrogens) and the samples were processed as described in Section 4.2. Inactivated controls (autoclaved sludge) were also monitored to check abiotic losses as mentioned in Section 4.2 . An aerobic degradation experiment was not carried out for PBDEs due to unavailability of the analytical standard in Brazil.

Anaerobic biodegradation experiments for triclosan, PAHs, and estrogens were carried out as described in Section 4.2.3 with the only difference being the incubation temperature and duration of the experiment. The experiment was not temperature controlled, but temperature was recorded using the same data logger employed in the aerobic experiment. The experiment was incubated for 15 days. Samples were processed as described in Section 4.2.4. The anaerobic experimental design for PBDEs proceeded differently. 25 ml of UASB sludge was added to a 50 ml amber glass vial and spiked at 30 µg/L of PBDEs in a solution of acetone (10 % v/v - this high amount was due to the poor solubility of PBDEs in acetone). Vials were

sealed with a 20-mm crimp cap with chlorobutyl vial stoppers and purged with nitrogen gas for 20 minutes to enable anaerobic conditions. Samples were processed similar to those of the other compounds above and analysed with the method described in Section 4.2.4. Surrogates for PBDE analysis were added at 10 ng/ml and 100 ng/ml for PCB-209 and 4PC-BDE-209 respectively before sample extraction.

Biodegradation experiments using the facultative pond inocula were carried out in 1 L Erlenmeyer flasks. 500 mL of sludge was added to the flasks followed by addition of chemicals (1000 µg/L for triclosan, 200 µg/L for PAHs and 100 µg/L for estrogens in the same reactor). Flasks were placed in an incubator equipped with two fluorescent tubes- 20W each (Marconi MA403) to study the effect of photolytic degradation. Some flasks were covered with aluminium, while others were exposed to the fluorescent light. Magnetic stirrers were used to ensure homogenous mixing and aeration of the sludge. The experiment was not temperature controlled but temperature was logged throughout. Dissolved oxygen (DO) and pH were monitored throughout the experiment to ensure those parameters were not limiting or extreme; they ranged from 6.1 – 7.0 mg/L DO and pH of 7.5 – 9.5 respectively. Sample processing and analysis was the same to that employed in the aerobic experiment. The batch tests were sampled at multiple points (day 1, 4, 7, 11, and 15) for 15 days. An inactivated control (autoclaved sludge) was also employed to check losses due to hydrolysis or volatilization (abiotic losses).

For PBDEs, sacrificial 10 ml COD tubes were used. 6 ml of facultative inocula was added to the reactors and PBDEs were spiked at 5 µg/L. The decision to use sacrificial tubes and the lower spiking concentration was due to the poor solubility of the PBDEs in water-miscible organic solvents (maximum solubility of the mix of PBDEs in acetone was 300 ng/ml). The tubes were placed in an incubator equipped with a shaker (Marconi MA420), and set at 140 rpm to ensure homogenous mixing and aeration of the sludge. Some of the tubes were covered with aluminium foil to account for non-photolytic degradation; others were left exposed for photolytic degradation. The experiment was not temperature controlled, but the temperature for the duration of the experiment was recorded with a temperature logger (Lascar Electronics, EasyLog EL-USB-2-LCD). An inhibition and abiotic control was also monitored to check abiotic and non-biological degradation losses. Triplicate samples (2 ml per sample) were collected in 2 ml Eppendorf tubes, spiked with surrogate standards (10 ng/ml and 100 ng/ml for PCB-209 and 4PC-BDE-209 respectively) and extracted with methods described in Section 3.1.2.

5.2.6 **Extraction and instrumental analysis for biodegradation experiment**

The compounds were extracted from the biosolids by SPE and were analysed by GC-MS, LC-MS/MS or GC-ECD. The protocol for extraction and analysis of triclosan and PAHs is described in Section 3.1.2, estrogens in (Coello-Garcia, 2018), and PBDEs in Section 3.2.2. SPE cartridges were stored at -20 ^{o}C in Brazil, then transported in boxes with cooling packs to the UK, where elution and instrumental analysis was performed.

5.2.7 **Microbial analysis**

Biosolids were stored at -20 ^{o}C immediately after sampling, and were transported to the UK a box with cooling packs. Samples were immediately stored back at -20 ^{o}C upon arrival in the UK until analysis. The detailed protocol for all the microbial analysis carried out for the biosolids from the biodegradation experiments was previously described in Section 4.3. In summary, genomic DNA (gDNA) was first extracted from the samples, generation of amplicon library by a fusion PCR method and finally sequenced on a Personal Genome Machine (PGM). Sequencing data analysis was done as described in Section 4.3.3.

5.2.8 **Statistical analysis**

Minitab, version 17 Statistical software (Minitab Inc., USA) was used to perform all statistical analysis. Variance analysis using one-way ANOVA was used to compare the biodegradation rate of the investigated chemicals under the applied treatment technology. Furthermore, STAMP was used to identify the change in bacterial community taxonomy before and after the aerobic or anaerobic treatment (Parks *et al.*, 2014). A two-sided G-test (w/ Yates') + Fisher's statistical test was carried in STAMP on each pair of sample to determine any significantly difference ($p < 0.05$) in the bacteria taxa.

5.3 Results and discussion

5.3.1 Characteristics and performance of the treatment plants

In terms of total suspended solids (TSS) removal, WWTP A was least effective, removing only 48 % as compared to 76 % and 75 % achieved by WWTP B and WWTP C respectively (Table 5-1). COD removal was also lowest in WWTP A with 42 % recorded when compared to 83 % and 80 % achieved by WWTP B and WWTP C respectively. Although the TSS and COD removal rates provided by the plant operator was 80 % and 82 % respectively. This discrepancy might be due to periodical variations in the loads that were not captured by grab sampling.

5.3.2 Assessing the fate and removal of micropollutants in the different WWTPs

5.3.2.1 Fate and extent of triclosan removal

The total (aqueous and particulate matter) concentration of triclosan in raw influent was 49.2 µg/L, 67.1 µg/L and 17.8 µg/L in WWTP A, WWTP B and WWTP C respectively (Table *5-2*). The total concentration of triclosan in the final effluent was 1.5 µg/L, 3.6 µg/L and 0.9 µg/L for WWTP A, WWTP B, and WWTP C respectively. These concentrations are similar to reported levels of triclosan in unfiltered municipal wastewater around the world (Table 5-3) (Kumar *et al.*, 2010; Lozano *et al.*, 2013).

In WWTP A, the concentration of triclosan in the aqueous phase decreased from 1303 ng/L to 893 ng/L (53 % removal) after primary treatment, then further reduced to 549 ng/L after secondary treatment (activated sludge treatment) (47 % removal)- thereby indicating removal in all phases of the treatment process (*Table 5-2*). Lozano *et al*. reported 75 % triclosan removal from the aqueous phase after primary treatment in an activated sludge WWTP (Lozano *et al.*, 2013), while another study reported up to 40 % triclosan removal (Thomas and Foster, 2005). About 97 % and 58 % triclosan removal was observed from the total and aqueous phases respectively after secondary treatment (*Figure 5-2*). This removal was most likely due to partitioning and settling out with sludge in the primary clarifier and biodegradation during secondary treatment (Lozano *et al.*, 2013).

Table 5-1 Characteristics of the selected WWTPs, including the concentrations of suspended solids and chemical oxygen demand across the different treatment stages

WWTP	Population (millions)	Treatment type (secondary)	TSS (mg/L)				VSS (mg/L)				COD (mg/L)			
			Sludge	**Influent**	**P Eff**	**S. Eff**	**Sludge**	**Influent**	**P. Eff**	**S. Eff**	**Sludge**	**Influent**	**P. Eff**	**S. Eff**
A	1.6	Activated sludge	4290 (210)	196 (25)	105 (12)	102 (12)	3750 (160)	161 (16)	86 (7)	65 (4)	1832 (79)	312 (12)	265 (12)	180 (9)
B	1.1	UASB + Trickling filters	22400 (790)	102 (9)	64 (3)	24 (2)	17640 (920)	100 (9)	55 (4)	24 (1)	997 (11)	252 (12)	112 (9)	43 (1)
C	0.015	WSP (1 AP, 1 FP)	75 (6)	208 (18)	121 (9)	52 (4)	73 (8)	103 (7)	109 (3)	50 (3)	111* (6)	456 (31)	91 (6)	90 (4)

- *n = 2 for COD measurements, n = 3 for total suspended solids (TSS) and volatile suspended solids (VSS) measurements*
- *Inf = Raw wastewater, P. Eff = primary effluent, S. Eff = secondary effluent, AP = anaerobic pond, FP = facultative pond*
- ** Sludge for WWTP C is wastewater taken from the top end of the facultative pond*
- *Values in parentheses are standard deviations*

In WWTP B, removal of triclosan from the aqueous phase was only observed after stage 1 secondary treatment (UASB treatment) as the concentration of triclosan was reduced by 60 % (*Figure 5-2*, *Table 5-2*). This indicates that the trickling filter system did not contribute to triclosan removal in this WWTP. About 95 % and 39 % of triclosan was removed from the total and aqueous phase respectively after treatment (*Table 5-2*). In WWTP C, 54 % of triclosan was removed after stage 1 secondary treatment (anaerobic pond treatment, and a further 19 % after stage 2 secondary treatment (facultative pond) (*Table 5-2*; Figure *5-2*). Considering the all phases, about 95 % triclosan removal was achieved by this WWTP.

Table 5-2 Concentration of triclosan in the influent, primary effluent and secondary effluent of three different WWTPs in Brazil

WWTP	**Influent (ng/L)**		**Primary effluent (ng/L)**		**Secondary effluent (ng/L)**		**Removal (%)**	
	Total	**Aqueous**	**Total***	**Aqueous**	**Total**	**Aqueous**	**Total removal**	**Aqueous removal**
A	49184 (7429)	1303 (223)	n.d	893 (37)	1486 (9)	549 (24)	97	58
B	67052 (720)	1262 (89)	n.d	756 (120)	3573 (1352)	775 (47)	95	39
C	17797 (2004)	1422 (39)	n.d	659 (32)	924 (78)	385 (23)	95	73

-Mean concentration presented with standard deviation in bracket
-Total represents triclosan concentration in the aqueous and particulate matter while Aqueous represents triclosan concentration in the aqueous phase alone.
- n.d represents not determined

About 92 – 98 % and 59 – 78 % of triclosan was present in the particulate phase of the influent and effluent samples respectively from the three WWTPs (*Figure 5-3*). Some previous studies reported about 75 – 80 % and 10 – 20 % of triclosan in influent and effluent respectively (Thomas and Foster, 2005; Lozano *et al.*, 2013). However, this partitioning might vary with different suspended solids concentrations especially in effluent samples (Thomas and Foster, 2005). Furthermore, this association with suspended solids was expected due to the high Log K_{ow} value (4.8) of triclosan (Kantiani *et al.*, 2008). Also, the relatively higher removal rates observed in the total liquid phase than in the aqueous phase might have been due to the high adsorption potential of triclosan, which promoted their adsorption to sludge across the treatment stream.

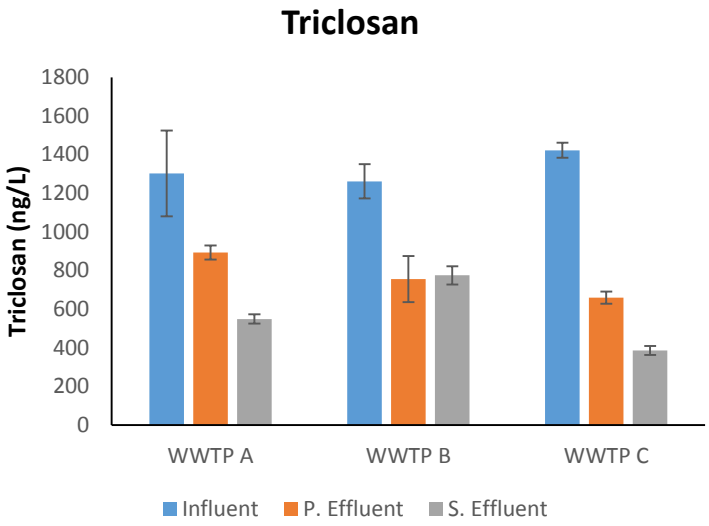


Figure 5-2 Concentration and distribution of triclosan in the aqueous phase of influent, primary effluent and secondary effluent for the three investigated WWTPs in Brazil

Triclosan effluent concentrations in the aqueous phase of WWTP A (549), WWTP B (775 ng/L and WWTP C (385 ng/L) exceeded the annual mean recommended standard of 100 ng/L for triclosan in freshwater and saltwater (UKTAG, 2013). The effluent concentration in these WWTPs was similar to reported levels in activated sludge WWTPs in Canada (Lee *et al.*, 2003), Australia (Kookana *et al.*, 2011), USA (Kumar *et al.*, 2010), Germany (Bester, 2005) and UK (Sabaliunas *et al.*, 2003) (Table 5-3). Hence, the concentration of triclosan is as high in low-middle income countries (LMICs) as in high income countries (HICs). No previous study has reported the levels of triclosan in effluents of UASB or WSP WWTPs. However, exceeding the recommended safety standard for triclosan indicates that WWTPs around the world (even with different treatment technologies) ineffective in removing triclosan in their current format.

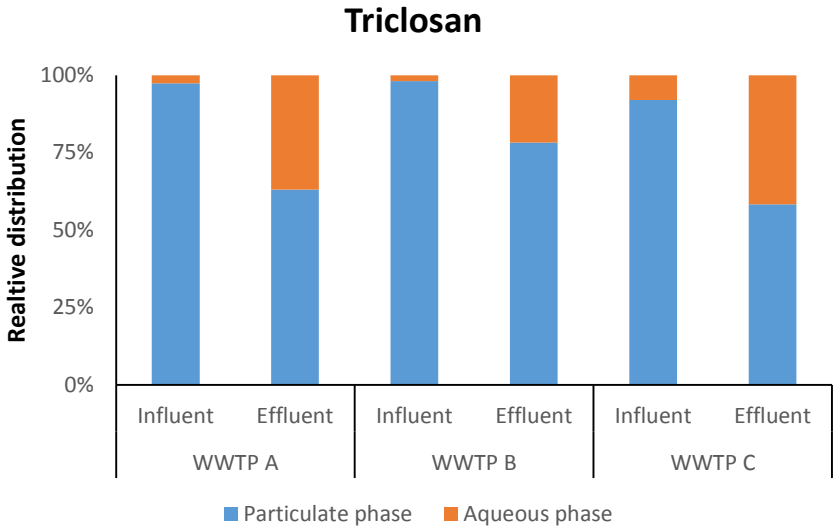


Figure 5-3 Partitioning of triclosan in the particulate and dissolved phases of influent and effluent samples from the three studied Brazilian WWTPs (WWTP A, B and C)

The concentration of methyl triclosan (a known biotransformation product of triclosan) was also monitored in the three WWTPs. Methyl triclosan was detected in influents, and this might be due to biotransformation of triclosan in the sewage network during transportation to the WWTPs (*Figure 5-4*). The concentration of methyl triclosan ranged from 138 – 468 ng/L in the three WWTPs. This was not surprising as methyl triclosan formation under aerobic and anoxic conditions has been reported, with the latter being relatively slower (Bester, 2005; Chen *et al.*, 2011). Removal of methyl triclosan in the WWTPs was discussed in Appendix G-8.8.

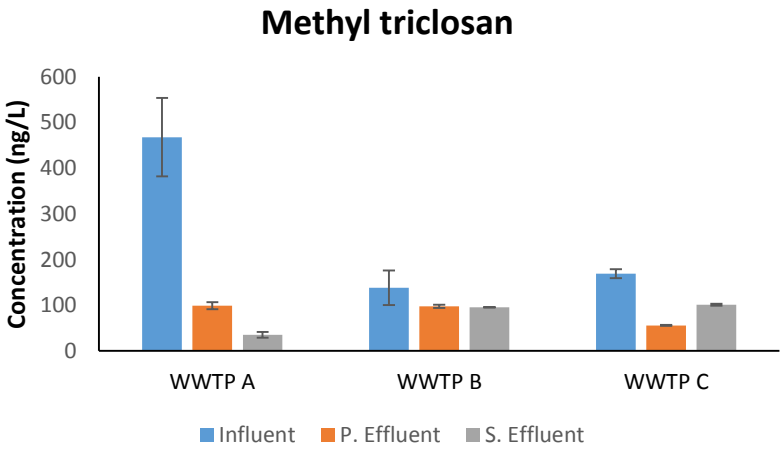


Figure 5-4 Concentration of methyl triclosan in the aqueous phase of influent, primary effluent and secondary effluent samples of the three WWTPs. P and S represents primary and secondary respectively. n = 3, standard deviation presented as error bars

Table 5-3 Concentration of triclosan and methyl triclosan in influent and effluent from WWTPs around the world.

Reference	Country	Triclosan (µg/L)		Methyl triclosan (µg/L)	
		Influent	Effluent	Influent	Effluent
This study	Brazil	17.8 – 67.1	0.9 – 3.6	n.d	n.d
		1.3 – 1.4*	0.4 – 0.6*	0.2 – 0.5*	0.04 – 0.1*
Lozano *et al.*, 2013	U.S.	8.1	0.9	0.03	0.006
Kumar *et al.*, 2013	U.S	5.4 – 85.2	0.2 - 5	n.d	n.d
		2.1 – 38.2*	0.2 – 4.8*	n.d	n.d
Sabaliunas *et al.*,, 2013	UK	7.5 – 21.9	0.3 – 1.1	n.d	n.d
Bester, 2005	Germany	4.8 – 7.3	0.3 – 0.6	0.002 – 0.003	0.008 – 0.015
Kokoona *et al.*, 2011	Australia	n.d	0.023 – 0.4*	n.d	n.d
Lee *et al.*, 2003	Canada	n.d	0.03 – 0.74*	n.d	n.d

**are concentrations in the aqueous phase only; n.d stands for not determined*

5.3.2.2 Fate and extent of PAH removal

The overall concentration of PAHs and their individual abundances in influent, primary effluent and secondary effluents from the three surveyed WWTPs are shown below (Table *5-4*, Table 5-5, Table 5-6) All PAHs except indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene and benzo(ghi)perylene were detected in all samples. The average total (aqueous and particulate matter) concentration of PAHs (sum of all detected PAHs) was highest in influent samples of WWTP C (29.3 µg/L) (Table 5-6) compared to 20.2 µg/L (Table 5-5) and 10.2 µg/L (*Table 5-4*) detected in WWTP B and WWTP A respectively. The total concentration of PAHs in effluent samples was higher in WWTP B (1.6 µg/L), than WWTP A (1.1 µg/L) and WWTP C (1.1 µg/L).

Low molecular weight (LMW) PAHs were the most abundant group of PAHs, followed by middle molecular weight (MMW) and high molecular weight PAHs in both influent and effluent samples. MMW PAHs have previously been reported to be the most abundant PAHs in raw wastewater, while LMW PAHs were the most abundant in the effluent (Busetti *et al.*, 2006), although this distribution mostly depends on the source of the PAHs entering the WWTP. The total concentration of PAHs in influents of the studied WWTPs detected in this study were similar to those reported in Spain (Sánchez-Avila *et al.*, 2009) and Greece (Manoli and Samara, 2008) , but two and ten times lower than those detected in China (Qi *et al.*, 2013) and Italy (Busetti *et al.*, 2006; Fatone *et al.*, 2011) respectively (Table 5-7). Naphthalene (Naph), phenanthrene (Phen), and benzo(b)fluoranthene (BbF) were the most abundant LMW, MMW and HMW PAHs respectively detected in the three WWTPs, which is in agreement with some other studies (Manoli and Samara, 2008; Wang *et al.*, 2013c).

Table 5-4 Concentration of 15 priority PAHs in influent and effluent of a Brazilian activated sludge WWTP (WWTP A)

Compound	Influent (ng/L)		Primary Effluent (ng/L)		Secondary Effluent (ng/L)		% total removal	% Aqueous removal
	Total	Aqueous	Total	Aqueous	Total	Aqueous		
Naph	2108.9 (86.0)	114.3 (6.0)	n.d	128.6 (4.4)	200.8 (13.4)	46.1 (4.0)	92.2	59.7
Acy	258.8 (11.3)	20.2 (0.7)	n.d	20.2 (0.5)	55.8 (0.5)	23.3 (0.9)	86.4	0.0
Ace	346.9 (11.4)	19.6 (0.4)	n.d	18.0 (0.4)	35.6 (1.4)	13.5 (0.6)	93.3	31.1
Flu	777.5 (15.3)	44.2 (0.8)	n.d	41.8 (1.6)	75.2 (9.1)	29.6 (2.2)	93.8	33.0
Phen	2118.8 (64.0)	65.7 (4.5)	n.d	63.4 (2.1)	140.3 (9.9)	43.1 (5.3)	95.3	34.4
Anth	395.3 (5.8)	26.6 (0.6)	n.d	26.7 (0.2)	67.0 (1.6)	28.2 (1.2)	89.5	0.0
Flt	1135.0 (21.6)	42.4 (1.0)	n.d	43.7 (1.3)	95.1 (7.6)	35.8 (2.8)	94.6	15.8
Pyr	1053.7 (10.8)	39.9 (1.4)	n.d	42.4 (1.3)	100.5 (6.1)	38.0 (2.4)	93.8	4.6
BaA	526.3 (9.7)	37.5 (0.3)	n.d	39.4 (0.4)	90.5 (0.4)	38.2 (1.7)	89.3	0.0
Chry	458.6 (5.0)	32.8 (0.3)	n.d	34.1 (0.6)	81.3 (0.5)	34.0 (0.9)	88.9	0.0
BbF	631.5 (18.5)	24.4 (0.4)	n.d	25.0 (1.2)	57.7 (1.1)	26.8 (2.5)	95.6	0.0
BaP	393.0 (10.7)	25.8 (0.3)	n.d	27.0 (0.5)	70.9 (3.4)	27.6 (3.6)	90.1	0.0
InPy	< MDL	< MDL	n.d	< MDL	< MDL	< MDL	n.d	n.d
DiahA	< MDL	< MDL	n.d	< MDL	< MDL	< MDL	n.d	n.d
BghiP	< MDL	< MDL	n.d	< MDL	< MDL	< MDL	n.d	n.d
∑LMW PAHs	6006.2	290.7	n.d	298.7	574.7	183.9	90.4	36.7
∑MMW PAHs	3173.6	152.6	n.d	159.5	367.4	146.0	88.4	4.3
∑HMW PAHs	1024.5	50.3	n.d	52.2	128.7	54.4	87.4	-8.2
∑PAHs	10204.3	553.5	n.d	575.5	1070.7	483.7	89.5	12.6

- PAHs abbreviation- naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (pyr), benz(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InPy), dibenz(a,h)anthracene (DiahA), and benzo(ghi)perylene (BghiP).
-Total concentration was not determined in primary effluent.
-n.d stands for 'not determined'; -LOQ stands for limit of quantification
-Mean concentration presented (n = 3) with standard deviation in bracket; -MDL represents method detection limit

The concentration of PAHs in the aqueous phase of the influent ranged from 554 ng/L to 1211 ng/L in the three WWTPs, while concentrations detected in effluent ranged from 431 ng/L to 484 ng/L. Influent concentrations were similar to those generally reported around the world (Cao *et al.*, 2005; Yao *et al.*, 2012; Qi *et al.*, 2013), but five to ten times lower than levels reported by Wang *et al*., in China (Wang *et al.*, 2013c) (Table 5-7).

There was no difference between the concentration of PAHs in both HICs and LMICs. Furthermore, the total effluent concentration from the WWTPs of those PAHs falling under the regulation of the EU WFD (naphthalene-Naph, anthracene- Anth, fluoranthene- Flt, benzo(b)fluoranthene- BbF, benzo(a)pyrene BaP) were below the mean allowable EQS concentrations for inland surface waters for fours PAHs, Naph, Anth, Flt, BaP (MAC-EQS values of 130, 000 ng/L, 100 ng/L, 120 ng/L, and 270 ng/L respectively), but were higher for BbF (17 ng/L) (EU, 2013a). The risks associated with discharging this effluent into water bodies is assessed in Section 5.3.3.6 below.

WWTP A achieved a total PAH removal of 90 %, and aqueous phase removal of 13 % (*Table 5-4*, *Figure 5-5*); with reductions observed mainly after secondary treatment (except for HMW PAHs). 92 % of total PAH removal was achieved by WWTP B, compared to the 33 % removal recorded from the aqueous phase (Table 5-5, *Figure 5-5*). In WWTP C, 96 % of PAHs were removed from the total phase, while 64 % were eliminated from the aqueous phase (Table 5-6, Figure 5-5). Increment in the concentration of PAHs after UASB treatment in WWTP (LMW PAHs) B, and anaerobic pond treatment in WWTP C (MMW PAHs) was observed. This was perhaps due to their residual concentrations already present in these treatment stages. The removal of PAHs from the studied WWTPs was most likely mainly via sorption onto solids in the settling tanks and reactors, with volatilization and biodegradation contributing less (Lei *et al.*, 2007; Liu *et al.*, 2011). Besides, volatilization has been reported to contribute to only about 1 – 2 % removal of PAHs in conventional wastewater treatment plants (Manoli and Samara, 2008). The higher removal observed in the total phase of the WWTPs suggests that removal was principally through sorption. To further explain this, about 90 – 97 % of LMW, MMW and HMW PAHs were present in the particulate phase of the influent samples from the three WWTPs, while 60 – 70 % of LMW to HMW PAHs partitioned onto the particulate matter in effluent samples (Figure 5-6). This is expected due to the high Log K_{ow} values of the PAHs that promotes their sorption onto solids (Sánchez-Avila *et al.*, 2009). WWTP C (WSP) was more effective in removing PAHs from the aqueous phase

- achieving 64 % removal compared to 13 % removal for WWTP A (activated sludge), and 33 % removal for WWTP B (UASB).

Table 5-5 Concentration of 15 priority PAHs in influent and effluent of a Brazilian UASB WWTP (WWTP B)

Compound	Influent (ng/L)		Primary Effluent (ng/L)		Secondary Effluent (ng/L)		% total removal	% Aqueous removal
	Total	Aqueous	Total	Aqueous	Total	Aqueous		
Naph	5519.1 (186.0)	217.5 (49.7)	n.d	318.6 (16.8)	299.0 (50.9)	102.8 (5.6)	94.6	52.7
Acy	495.5 (11.6)	24.2 (2.1)	n.d	23.3 (0.8)	66.6 (5.7)	20.1 (0.2)	86.6	16.9
Ace	1255.9 (14.8)	33.8 (4.7)	n.d	34.4 (0.2)	124.6 (14.5)	15.6 (0.5)	90.1	53.9
Flu	2170.8 (63.8)	75.7 (13.6)	n.d	81.9 (4.4)	225.9 (30.1)	36.3 (0.4)	89.6	52.1
Phen	4296.4 (140.6)	102.6 (32.1)	n.d	99.4 (6.0)	196.2 (28.0)	68.7 (0.5)	95.4	33.0
Anth	472.1 (56.4)	29.6 (3.1)	n.d	29.1 (0.4)	71.5 (2.8)	26.7 (0.2)	84.9	9.9
Flt	2115.5 (84.8)	49.5 (8.7)	n.d	47.7 (1.9)	111.1 (9.7)	43.4 (1.4)	94.7	12.4
Pyr	1574.2 (65.8)	45.3 (7.0)	n.d	42.8 (0.9)	113.1 (6.4)	40.4 (0.4)	92.8	10.7
BaA	545.4 (35.2)	37.7 (1.2)	n.d	38.3 (0.2)	113.8 (30.3)	36.5 (0.1)	79.1	3.2
Chry	471.0 (20.9)	32.8 (0.8)	n.d	32.8 (0.2)	92.8 (17.3)	31.9 (0.1)	80.3	2.8
BbF	706.7 (56.3)	24.5 (1.0)	n.d	25.5 (0.6)	81.2 (14.1)	24.6 (0.1)	88.5	0.0
BaP	567.7 (50.7)	26.3 (0.8)	n.d	25.5 (0.5)	76.3 (5.4)	25.4 (0.8)	86.6	3.3
InPy	< MDL	< MDL	n.d	< MDL	< MDL	< MDL	n.d	n.d
DiahA	< MDL	< MDL	n.d	< MDL	< MDL	< MDL	n.d	n.d
BghiP	< MDL	< MDL	n.d	< MDL	< MDL	< MDL	n.d	n.d
∑LMW PAHs	14209.8	483.5	n.d	586.6	983.9	270.3	93.1	44.1
∑MMW PAHs	4706.1	165.3	n.d	161.6	430.7	152.1	90.8	7.9
∑HMW PAHs	1274.4	50.8	n.d	51.0	157.5	50.0	87.6	1.7
∑PAHs	20190.3	699.6	n.d	799.3	1572.0	472.4	92.2	32.5

PAHs abbreviation- naphthalene (Naph), acenaphthylene (Acy), Acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (pyr), benz(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InPy), dibenz(a,h)anthracene (DiahA), and benzo(ghi)perylene (BghiP).

-Total concentration was not determined in primary effluent.

-n.d stands for 'not determined'; - LOQ stands for limit of quantification

-Mean concentration presented (n = 3) with standard deviation in bracket; -MDL represents method detection limit

Table 5-6 Concentration of 15 priority PAHs in influent and effluent of a Brazilian WSP WWTP (WWTP C) -Mean concentration presented (n = 3) with standard deviation in bracket

Compound	Influent		Primary Effluent (ng/L)		Secondary Effluent (ng/L)		% total removal	% Aqueous removal
	Total	Aqueous	Total	Aqueous	Total	Aqueous		
Naph	3674.3 (92.7)	435.8 (24.9)	n.d	231.6 (14.5)	124.7 (1.2)	61.4 (2.1)	96.6	85.9
Acy	1561.9 (14.1)	31.7 (1.3)	n.d	24.9 (1.1)	47.6 (1.2)	19.1 (0.2)	97.0	39.8
Ace	770.8 (11.4)	47.8 (3.3)	n.d	28.9 (2.0)	34.6 (1.3)	15.6 (0.4)	95.5	67.3
Flu	3987.7 (30.0)	232.1 (11.7)	n.d	140.7 (4.6)	68.2 (1.4)	36.7 (1.9)	98.3	84.2
Phen	7658.8 (73.3)	248.6 (16.1)	n.d	176.3 (9.9)	170.6 (6.9)	60.1 (1.3)	97.8	75.8
Anth	2073.3 (46.7)	38.2 (1.3)	n.d	33.4 (0.2)	71.2 (3.9)	26.8 (0.3)	96.6	30.0
Flt	991.4 (17.5)	44.8 (3.3)	n.d	50.9 (0.9)	127.5 (1.8)	46.6 (3.5)	87.1	0.0
Pyr	4073.5 (46.2)	62.4 (3.1)	n.d	67.5 (1.5)	113.8 (3.5)	41.4 (1.6)	97.2	33.6
BaA	723.3 (12.1)	36.5 (0.6)	n.d	37.7 (0.3)	91.5 (2.1)	35.9 (0.3)	87.4	1.6
Chry	666.3 (31.4)	32.9 (0.6)	n.d	34.0 (0.5)	82.8 (4.9)	32.4 (0.6)	87.6	1.4
BbF	541.4 (26.9)	25.5 (0.6)	n.d	26.5 (0.9)	68.8 (13.2)	27.5 (2.5)	87.3	0.0
BaP	573.4 (22.7)	25.1 (0.4)	n.d	28.1 (1.3)	66.3 (6.0)	27.4 (1.8)	88.4	0.0
InPy	< MDL	< MDL	n.d	< MDL	< MDL	< MDL	n.d	n.d
DiahA	< MDL	< MDL	n.d	< MDL	< MDL	< MDL	n.d	n.d
BghiP	< MDL	< MDL	n.d	< MDL	< MDL	< MDL	n.d	n.d
∑LMW PAHs	19726.7	996.0	n.d	635.7	516.9	219.8	97.4	77.9
∑MMW PAHs	6454.5	145.4	n.d	190.1	415.6	156.3	93.6	-7.5
∑HMW PAHs	1114.8	69.4	n.d	54.6	135.1	54.9	87.9	20.8
∑PAHs	27296.0	1210.8	n.d	880.4	1067.6	431.1	96.1	64.4

PAHs abbreviation- naphthalene (Naph), acenaphthylene (Acy), Acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (pyr), benz(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InPy), dibenz(a,h)anthracene (DiahA), and benzo(ghi)perylene (BghiP).

-Total concentration was not determined in primary effluent.

-n.d stands for 'not determined'; - LOQ stands for limit of quantification

-Mean concentration presented (n = 3) with standard deviation in bracket; -MDL represents method detection limit

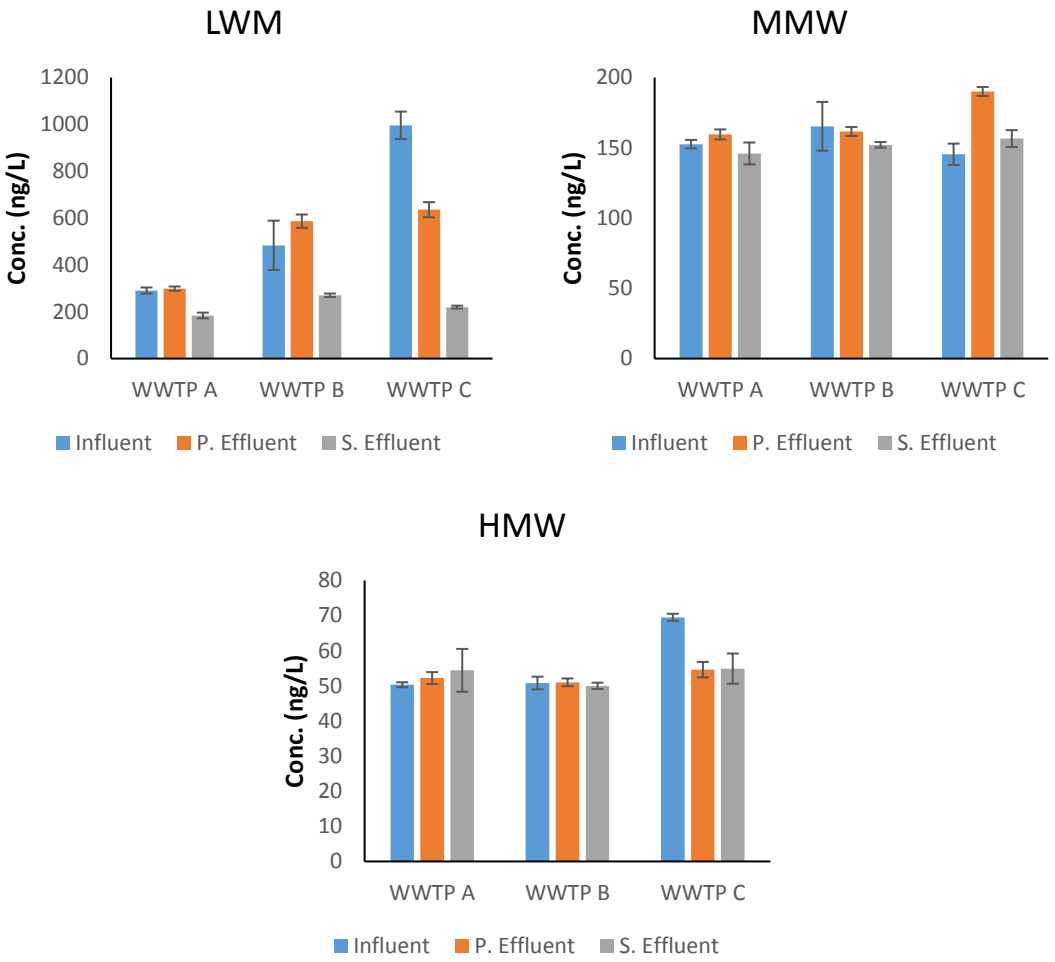


Figure 5-5 Concentration and distribution of PAHs in the aqueous phase of influent, primary effluent and secondary effluent of the three studied Brazilian WWTPs. P and S refers to primary and secondary respectively

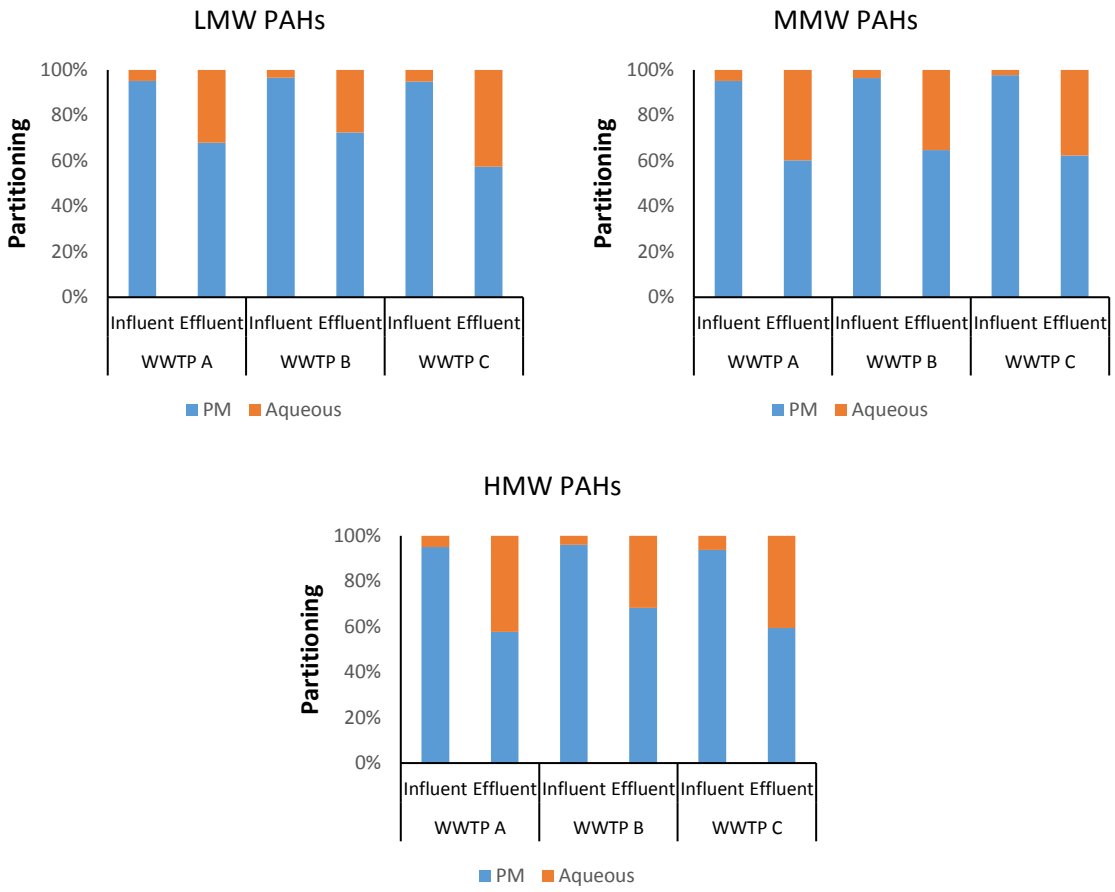


Figure 5-6 Partitioning of LMW PAHs, MMW PAHs and HMW PAHs in the particulate and dissolved phases of influent and effluent samples from the three Brazilian WWTPs

Table 5-7 Concentration of PAHs in influent and effluent of WWTPs around the world

Reference	Country	PAHs in total phase (µg/L)		PAHs in aqueous phase (µg/L)	
		Influent	Effluent	Influent	Effluent
This study	Brazil	10.2 – 27.3	1.1 – 1.6	0.6 – 1.2	0.4 – 0.5
Sanchez-Avila *et al.*, 2009	Spain	14.3	3.9	n.d	n.d
Manoli and Samara, 2008	Greece	10.6	6.6	n.d	n.d
Qi *et al.*, 2013	China	1.5 – 5.0	0.2 – 0.4	0.6 – 2.0	0.2 – 0.4
Wang *et al.*, 2013	China	n.d	n.d	5.8	2.2
Cao *et al.*, 2005	China	n.d	n.d	n.d	0.4
Fatone *et al.*, 2011	Italy	0.1 – 1.5	0.1 – 0.2	n.d	n.d
Busetti *et al.*, 2006	Italy	3.8	1.1	0.2	0.07

-n.d stands for not determined

5.3.2.3 Fate and extent of estrogen removal

Only E1 and E3 were detected in these WWTPs, while E2 and EE2 were below the method detection limit (0.5 ng/L) (*Table 5-8*). E3 was the most abundant estrogen in the influent of all the WWTPs, with an aqueous phase concentration of 1234 ng/L, 1548 ng/L and 625 ng/L in WWTP A, WWTP B and WWTP C respectively. E3 has been reported to be the most abundant estrogen in wastewater around the world (Kim *et al.*, 2007; Coleman *et al.*, 2010) as they are excreted the most by humans (Ternes and Joss, 2007). However, the E3 concentrations in influent reported in this study were relatively high compared to globally recorded concentrations (10 – 1100 ng/L; Table 5-9) (Kim *et al.*, 2007; Gabet-Giraud *et al.*, 2010; Luo *et al.*, 2014; Liu *et al.*, 2015).

The aqueous concentration of E1 in WWTP A, WWTP B and WWTP C was 78 ng/L, 107 ng/L and 51 ng/L respectively (*Table 5-8*). These results differ from the reported occurrence and concentrations of estrogens (E1, E2 and EE2) by Pessoa et al. (Pessoa *et al.*, 2014) in Brazilian WWTPs with identical technologies and process configurations to those reported in this study. These authors detected E1, E2 and EE2 in influent samples but did not measure E3 concentrations. They also suggested that E1 was more frequently detected in influents due to partial degradation of E2 to E1, and de-conjugation of E1 conjugated compounds (E1 sulfonide or glucuronide) in sewer systems. This assertion was further supported by E1 concentrations in the influent that were five times higher than E2. Therefore, relatively low levels of E1 in this study, and the reported E2 degradation might be responsible for non-detection of E2 in the examined WWTPs.

Furthermore, some studies reported a five to ten times higher concentration of E1 in influents from Brazilian wastewater plants (Froehner *et al.*, 2010; Pessoa *et al.*, 2014) in comparison with our results. These extremely high concentrations of estrogens reported by (Froehner *et al.*, 2010) and (Pessoa *et al.*, 2014) are unlikely to be achieved in WWTPs - especially for EE2 (Table 5-9), unless a huge load of this chemical were discharged into this WWTP during this period by pharmaceutical companies post production. Considering that in 2017, there was 56 million women within the reproductive age (15 – 49 years old) in Brazil (Indexmundi, 2017), and 14 % of them used the contraceptive pill (UN, 2017). The maximum EE2 usage per head in Brazil will be 1.92 µg/d; of which 0.78 µg/d is excreted (based on 26 µg/d of EE2 ingested even in industrialized countries (Johnson and Williams, 2004)). Therefore, using the information of population served and daily flow in both (Froehner *et al.*, 2010) and (Pessoa *et al.*, 2014), the maximum concentration of EE2 in both plants should be around 8.9 – 9.2 ng/L (assuming all the EE2 ingested by the women was excreted; see Appendix D- 8.5 for

calculation), and is 3 – 4 orders of magnitude lower than their reported concentration (up to 3180 ng/L) (Table 5-9). However, Queiroz *et al.* could not detect E2 and EE2 above the LODs (9.3 and 12.4 ng/L respectively) from influent samples from one of the same WWTPs (WWTP A) used in this study (Queiroz *et al.*, 2012). Coleman *et al.*, also reported that E2 and EE2 concentrations in WWTP influent in Australia was below their method detection limits (1 and 0.1 ng/L respectively) (Coleman *et al.*, 2010). Also, the E1 influent concentrations in this study are similar to those reported around the world (Gabet-Giraud *et al.*, 2010; Salgado *et al.*, 2010) (Table 5-9). E1 is considered as one of the most environmentally important estrogens because of its more frequent detection at higher concentrations compared to the other estrogens, and can be used to measure estrogenicity in the environment despite its lower potency compared to E2 (Coleman *et al.*, 2010).

Table 5-8 Concentration of E1 and E3 in the influent, primary effluent and secondary effluent of the three studied Brazilian WWTP

Compound	**E1 (ng/L)**				**E3 (ng/L)**			
	Influent	**P. Effluent**	**S. Effluent**	**% Removal**	**Influent**	**P. Effluent**	**S. Effluent**	**% Removal**
WWTP A	77.8 (3.3)	65.3 (5.4)	16.0 (2.0)	79.4	1233.7 (17.1)	1068.7 (51.8)	64.3 (9.1)	94.8
WWTP B	106.5 (6.6)	151.8 (10.2)	11.9 (1.4)	88.9	1547.9 (148.8)	1254.5 (641.3)	12.5 (1.0)	99.2
WWTP C	50.5 (5.4)	130.2 (29.4)	0.1 (0.08)	99.7	625.1 (26.4)	3814 (559.8)	13.0 (1.7)	97.9

P and S represents primary and secondary effluent; standard deviation in bracket

The mean removal of E1 from the aqueous phase was 79 %, 89 % and 99 % in WWTP A, WWTP B and WWTP C respectively. Whereas, E3 removal was 95 %, 99 % and 98 % for WWTP A, WWTP B and WWTP C respectively. In WWTP A, 17 % and 14 % of E1 and E3 was removed after primary treatment respectively, while secondary treatment removed 62 % and 81 % (*Table 5-8*). This confirms reports that activated sludge secondary treatment is effective in removing these estrogens (Leusch *et al.*, 2006), and the removal rates achieved were similar those reported previously (Salgado *et al.*, 2010; Nie *et al.*, 2012; Pessoa *et al.*, 2014). However, it is the final effluent concentration, not percentage removal and how it compares to the PNEC values that are important for determining risk to the environment.

In WWTP B, the concentration of E1 increased from 107 ng/L to 152 ng/L after primary treatment (UASB treatment), before reducing to 12 ng/L after secondary treatment (trickling filters). For E3, primary treatment removed 19 %, and secondary treatment removed a further 80 %. This increased concentration after primary treatment might be due to accumulation of E1 in the UASB reactor before sampling - since grab samples did not take into account the

hydraulic retention time and hence may not correctly represent the treatment efficiency achieved. Pessoa *et al.* (Pessoa *et al.*, 2014) reported E1 removal below their detection limits (35.4 ng/L) in a WWTP utilizing UASB technology equipped with chlorine post-treatment (that may have also contributed to E1 removal). Froehner *et al.*, also reported E1 removal below their detection limits (values not reported) from a WWTP using UASB treatment with an attached dissolved air flotation reactor (Froehner *et al.*, 2010). Hence, the efficiency of the UASB system alone could not be determined from these previous studies. In this study, it was the trickling filter post-UASB treatment that removed the majority of the E1 and E3. Trickling filter systems have been reported to possess a similar E1 removal capacity as activated sludge system, with an achieved removal of 90 – 95 % (Salgado *et al.*, 2010). Although some studies have shown they are ineffective (Liu *et al.*, 2015) .There is no report of E3 removal by UASB systems in literature, but up to 90 % removal has been reported in trickling filter systems (Gabet-Giraud *et al.*, 2010).

Table 5-9 Concentration of estrogens in influent and effluent of WWTPs around the world

Reference	**Country**	**E3 (ng/L)**		**E1 (ng/L)**		**E2 (ng/L)**		**EE2 (ng/L)**	
		Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
This study	Brazil	625 - 1548	12.5 – 64.3	50.5 - 107	0.1 - 16	<MDL	<MDL	<MDL	<MDL
Gabet-Giraud *et al.*, 2010	France	26.8 - 658	5.2 – 47.7	18.8 - 170	0.1 - 58	5.1 – 37.9	0.5 – 11.9	<10	1.6 – 4.6
Salgado *et al.*, 2010	Portugal	n.d	n.d	189 - 2484	25	344	<1	103 – 106	<1
Liu *et al.*, 2015	China	53.9 - 470	0.2 – 47.5	38.6 - 427	0.8 - 116	17.9 – 64.8	1.4 – 28.1	n.d	n.d
Liu *et al.*, 2015	US	19 - 167	0.2 - 5	2.6 – 66.7	0.9 – 22.3	1 – 57.6	0.2 – 6.4	n.d	n.d
Liu *et al.*, 2015	Italy	31 - 129	0.4 - 15	11 - 71	0.3 – 44.6	9.5 - 25	0.8 – 3.7	n.d	n.d
Coleman *et al.*, 2010	Australia	120 - 270	60 - 160	30 - 75	<1 - 10	<1	<1	<0.1	<0.1
Pessoa *et al.*, 2014	Brazil	n.d	n.d	<118 - 3050	<118 - 2080	<38 - 776	<38 - 397	<1 - 3180	<1 - 176
Froehner *et al.*, 2011	Brazil	n.d	n.d	870 - 1380	<MDL	1330 - 2270	490 - 760	600 - 1260	<MDL - 470

MDL represents method detection limit

In WWTP C that utilizes waste stabilization pond technology, the concentration of E1 and E3 increased after anaerobic pond treatment and was removed by a high degree after facultative pond treatment (*Table 5-8*). This interesting increase in E1 and E3 concentration may be due to previous accumulation of these compounds in the anaerobic pond before sampling as explained previously. Although deconjugation of E1 and E3 conjugated compounds might also have contributed to this (Pessoa *et al.*, 2014). A similar occurrence of increased E3

concentration after waste stabilization pond treatment was reported in another study (Coleman *et al.*, 2010). WWTP C recorded the highest removal of E1 (99 %) when compared to WWTP A and WWTP B. This removal was consistent with those reported by Froehner *et al.* by WSPs (Froehner *et al.*, 2010), but was higher than the 34-82 % reported by Coleman et al., (Coleman *et al.*, 2010) and 31 – 62 % reported by Pessoa et al., (Pessoa *et al.*, 2014). The 98 % E3 removal achieved by the WWTP was also higher than the 26 – 36 % reported in another study (Coleman *et al.*, 2010). The relatively poor removal reported by Pessoa *et al*., and Coleman *et al*., might be due the different system configuration (absence of an anaerobic pond and presence of a maturation pond). However, the most important quantities are the effluent concentrations and how they compare to PNEC values. The better estrogen removal rate achieved in WWTP C compared to WWTP A and WWTP B might be due to the photo-oxidizing effect of the WSP, which may have contributed to estrogen removal. This is due to increased photo-oxidative potential of the ponds because of the high oxygen concentrations and presence of humic substances (Davies-Colley *et al.*, 1999). Furthermore, Coleman *et al*., reported complete photo-degradation of E1 and E3 under UV light in their studies (Coleman *et al.*, 2010).

The effluent concentration of E1 ranged from 0.1 ng/L for WWTP C to 16 ng/L for WWTP A. These concentrations were similar to those reported around the world (Luo *et al.*, 2014; Liu *et al.*, 2015), but were ten times lower than those reported by other studies in Brazil (Pessoa *et al.*, 2014) (Table 5-9). The concentration of E3 in effluent also ranged from 13 ng/L for WWTP C to 64 ng/L for WWTP A. These concentration were similar to those reported in France (Gabet-Giraud *et al.*, 2010), but over ten times higher than those reported in Korea (Behera *et al.*, 2011), China (Nie *et al.*, 2012), and two to five times lower than reported levels in another study in Brazil (Coleman *et al.*, 2010) (Table 5-9). Caldwell *et al*. estimated the predicted no effect concentration (PNEC) values in aquatic organisms for E1 and E3 to be 6 ng/l and 60 ng/L respectively (Caldwell *et al.*, 2012). Only the effluent from WWTP C was below the PNEC value for E1 in this study. For E3, the effluent from WWTP B and WWTP C were below the PNEC value, while effluent from WWTP A exceeded this level. Exceeding this PNEC values indicates the ineffectiveness of WWTP A and WWTP B to remove E1, and E3 for WWTP C in their current format, although river dilution might ensure compliance.

5.3.2.4 Fate and extent of PBDE removal

BDE 47, 100 and 209 were detected in influent samples of the three WWTPs, BDE 183 was detected in WWTP A and WWTP B, BDE 154 was detected in WWTP B and WWTP C, and BDE 99 was only detected in WWTP C (Table *5-10*, Table *5-11*, Table 5-12). Only BDE 28 was undetected in the influent samples from all plants. The total (aqueous and particulate matter) concentration of each BDE was between 13 – 103.2 ng/L (∑PBDE = 194 ng/L) for WWTP A, 16 – 143 ng/L (∑PBDE = 222 ng/L) for WWTP B, and 18 – 251 ng/L (∑PBDE = 270 ng/L) for WWTP C. To the best of author's knowledge, this is the first time that PBDEs have been reported in Brazilian wastewater treatment plants. Furthermore, these determined concentrations are similar to reported levels around the world (Clarke *et al.*, 2010; Kim *et al.*, 2013; Xiang *et al.*, 2014) (Table 5-13).

Table 5-10 Concentration of BDE congeners in the influent, primary effluent and secondary effluent of WWTP A

Compound	**Influent (ng/L)**		**P. Effluent (ng/L)**		**S. Effluent (ng/L)**		**Removal (%)**	
	Total	Aqueous	Total	Aqueous	Total	Aqueous	% Total removal	% Aqueous removal
BDE 28	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	-	-
BDE 47	44.0 (6.0)	9.3 (0.1)	<MDL	13.4 (2.3)	3.5 (0.9)	1.5 (0.2)	92.0	62.4
BDE 99	<MDL	<MDL	<MDL	-<MDL	<MDL	0.85 (0.1)	-	-
BDE 100	33.5 (4.5)	3.9 (0.2)	<MDL	6.7 (0.6)	<MDL	<MDL	100.0	100.0
BDE 153	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	-	-
BDE 154	<MDL	<MDL	<MDL	<MDL	7.5 (0.2)	1.2 (0.2)	-	-
BDE 183	13.1 (2.4)	<MDL	<MDL	<MDL	<MDL	<MDL	100.0	-
BDE 209	103.2 (22.2)	13.2 (0.2)	<MDL	12.9 (0.8)	<MDL	<MDL	100.0	100.0
∑PBDE	193.8	26.4	<MDL	33	11.0	3.6	98.1	86.4
∑PBDE EU WFD	-	-	-	-	11	3.6	85.8	72.7

-P. and S. represents primary and secondary respectively; MDL represents method detection limit; standard deviation in bracket

About 98 %, 86 % and 95 % PBDE (∑PBDE) removal from the total phase was achieved by WWTP A, WWTP B and WWTP C respectively. WWTP C achieved a higher removal of PBDEs (94.1 %) from the aqueous phase compared to the 73 % and 43 % achieved by WWTP A and WWTP B respectively. 90 % and 22 % of PBDEs were removed after stage 1 secondary treatment in WWTP B and WWTP C, while the concentration PBDEs increased after primary treatment in WWTP A. The increase in the aqueous concentration of individual PBDE congeners (BDE 47, 100 in WWTP A, BDE 47, 100, 183 in WWTP B, and BDE 47 in

WWTP C) after primary treatment may be a as result of gradual release of the particulate matter-bound compounds in the influent samples into the dissolved/aqueous phase. Furthermore, this might also be due to the accumulation of these compounds in the primary treatment process before grab-sampling. Hence, these post-primary treatment concentrations may not have been representative of the levels in the raw influent. Some other authors have reported increased level of BDE 47, and 100 after primary treatment (Clarke *et al.*, 2010) and secondary treatment (Daso *et al.*, 2012) in conventional wastewater treatment plants.

Table 5-11 Concentration of BDE congeners in the influent, primary effluent and secondary effluent of WWTP B

Compound	Influent (ng/L)		P. Effluent (ng/L)		S. Effluent (ng/L)		Removal (%)	
	Total	Aqueous	Total	Aqueous	Total	Aqueous	% Total removal	% Aqueous removal
BDE 28	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	-	-
BDE 47	41.9 (3.1)	12.8 (1.3)	<MDL	20 (2.0)	4.7 (1.4)	4.1 (0.4)	88.8	68.0
BDE 99	<MDL	<MDL	<MDL	1.3 (0.1)	<MDL	1.3 (0.1)	-	-
BDE 100	21.3 (4.3)	1.9 (0.1)	<MDL	2.5 (0.1)	<MDL	<MDL	100.0	100.0
BDE 153	16.2 (3.9)	<MDL	<MDL	<MDL	2.1 (0.1)	<MDL	87.0	-
BDE 154	<MDL	4.5 (2.0)	<MDL	15.6 (3.9)	3.4 (0.5)	5.6 (0.3)	-	0.0
BDE 183	<MDL	19.1 (2.3)	<MDL	15.4 (3.5)	9.8 (3.7)	3.8 (0.2)	-	80.1
BDE 209	143.0 (21.8)	45.3 (7.0)	<MDL	10.0 (1.2)	12.1 (0.1)	<MDL	91.5	100.0
∑PBDE	222.4	83.6	<MDL	64.8	32.1	14.8	85.6	82.3
∑PBDE EU WFD	-	-	-	-	10.2	11.0	87.2	42.7

-*P. and S. represents primary and secondary respectively; MDL represents method detection limit; standard deviation in bracket*

The total concentration of PBDE in the effluent of the three studied WWTPs was between 11 – 32 ng/L (∑PBDE EU WFD congeners = 10 - 13 ng/L). These concentrations are an order of magnitude lower than the EU WFD MAC-EQS and are similar to reported levels in Canada (Kim *et al.*, 2013), Australia (Clarke *et al.*, 2010) and China (Xiang *et al.*, 2014; Deng *et al.*, 2015) (Table 5-13). BDE 209 has been reported to dominate the total concentrations of PBDEs founds in wastewater around the world (Peng *et al.*, 2009; Cristale and Lacorte, 2015). This was also the case in Brazilian wastewaters in this studies as BDE 209 accounted for 53 %, 64 % and 93 % of total PBDE concentration in the influent of WWTP A, WWTP B and WWTP C respectively. BDE 209 was not detected in the effluent of WWTP A and WWTP C, but was found in the effluent of WWTP B at a concentration that will pose risk on aquatic organisms – estimated Risk Quotient of 2.5 according to Cristale *et al*. (Cristale *et al.*,

2013). Due to the high Log K_{ow} of PBDEs, sorption onto solids and subsequent removal during primary and secondary treatment is expected (Sánchez-Avila *et al.*, 2009). However, biodegradation can also play a major role in their removal, and their biodegradability was studied in Section 5.3.3. Biodegradation of PBDEs can occur under both aerobic and anaerobic conditions (Xia, 2013).

Table 5-12 Concentration of PBDE congeners in the influent, primary effluent and secondary effluent of WWTP C

Compound	Influent (ng/L)		P. Effluent (ng/L)		S. Effluent (ng/L)		Removal (%)	
	Total	Aqueous	Total	Aqueous	Total	Aqueous	% Total removal	% Aqueous removal
BDE 28	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	-	-
BDE 47	<MDL	2.6 (0.3)	<MDL	9.8 (2.8)	3.9 (0.7)	2.9 (0.5)	-	0.0
BDE 99	<MDL	4.0 (0.9)	<MDL	1.3 (0.1)	3.1 (0.6)	2.8 (0.3)	-	30.0
BDE 100	18.4 (0.1)	<MDL	<MDL	<MDL	1.5 (0.1)	<MDL	91.8	-
BDE 153	<MDL	<MDL	<MDL	<MDL	2.6 (0.2)	1.3	-	-
BDE 154	<MDL	4.5 (0.9)	<MDL	1.5 (0.2)	1.7 (0.4)	1.1 (0.3)	-	75.6
BDE 183	<MDL	<MDL	<MDL	1.7 (0.3)	1.0 (0.1)	0.7 (0.1)	-	-
BDE 209	251.2 (20.4)	136.9	<MDL	<MDL	<MDL	<MDL	-	-
∑PBDE	269.6	148.0	<MDL	14.3	13.8	8.8	94.9	94.1
∑PBDE EU WFD	-	-	-	-	12.8	8.1	30.4	27.0

P. and S. represents primary and secondary respectively; MDL represents method detection limit; standard deviation in bracket

Table 5-13 Concentration of total PBDEs in influent and effluent of WWTPs around the world

Reference	Country	PAHs in total phase (ng/L)		PAHs in aqueous phase (ng/L)	
		Influent	Effluent	Influent	Effluent
This study	Brazil	198 - 269	11.1 – 32.2	26.4 - 148	3.6 – 14.8
Clark *et al.*, 2009	Australia	42 - 100	0.14 – 0.71	n.d	n.d
Kim et al., 2013	Canada	20 - 1000	1 - 250	n.d	n.d
Xiang *et al.*, 2014	China	5.3 – 27.9	1.5 – 7.7	1.0 – 13.6	0.5 – 3.4
Peng *et al.*, 2013	China	566	4	n.d	n.d
North *et al.*, 2004	U.S.	n.d	29.1	n.d	n.d
Daso *et al.*, 2012	South Africa	310 - 2720	102 – 9800	n.d	n.d
Busetti *et al.*, 2006	Italy	3.8	1.1	0.2	0.07

-n.d stands for not determined

5.3.3 Biodegradation of the selected micropollutants under different redox conditions

5.3.3.1 Biodegradation of triclosan under different redox conditions and inocula

5.3.3.1.1 Biotransformation of triclosan with activated sludge inocula under aerobic conditions

Under aerobic conditions, triclosan concentration in the batch tests decreased by 74 % over the duration of the experiment (168 hours) (Figure 5-7). Chen *et al*., reported a similar observation of 86 % degradation of triclosan spiked at 500 µg/L after 168 hours (Chen *et al.*, 2011). The biodegradation of triclosan was assumed to follow first order kinetics (R^2 = 0.78) with a rate constant of 0.0072 h^{-1} and an estimated half-life of 96 hours (Figure 5-7), although a scatter around first order kinetics and distinct multiple phases were observed. This rate is similar to those reported by (Chen *et al.*, 2011) , but slower than those reported by (Armstrong *et al.*, 2018) –perhaps due to the two folds lower starting concentration (Table 5-14).

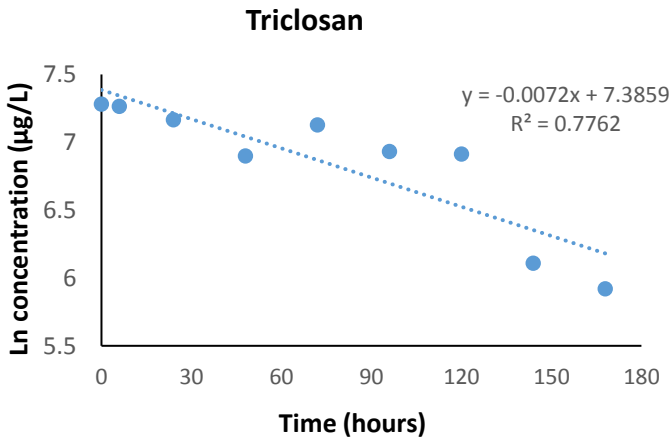


Figure 5-7 Degradation of triclosan with activated sludge inocula under aerobic conditions

Table 5-14 First order degradation rates of triclosan with activated sludge inocula in different studies

Inoculum	Temp. (°C)	Concentration spiked	Rate (k) (h^{-1})	S_k	Half-life ($t_{1/2}$) (h)	References
Activated sludge (MLSS 3.3 g/L)	20	1000 µg/L	0.0072	0.0015	96	This study
Activated sludge (MLSS 4.0 g/L)	17	500 µg/L	0.0095	-	73	Chen *et al*., 2011
Activated sludge (MLSS 5.0 g/L)	21	23 µg/g	0.0170	-	41	Armstrong *et al*., 2018

k represents the first order rate constant, S_k represents standard error of the rate constant, and $t_{1/2}$ represents the half-life.

Methyl triclosan was produced in the batch tests, and concomitantly increased with decreasing triclosan concentration (Figure 5-8), thereby supporting reports that methyl triclosan is biotransformation product of triclosan under aerobic conditions (Heidler and Halden, 2007). Methyl triclosan is known to more persistent, bio-accumulative and lipophilic than triclosan (Lindström *et al.*, 2002; Balmer *et al.*, 2004).

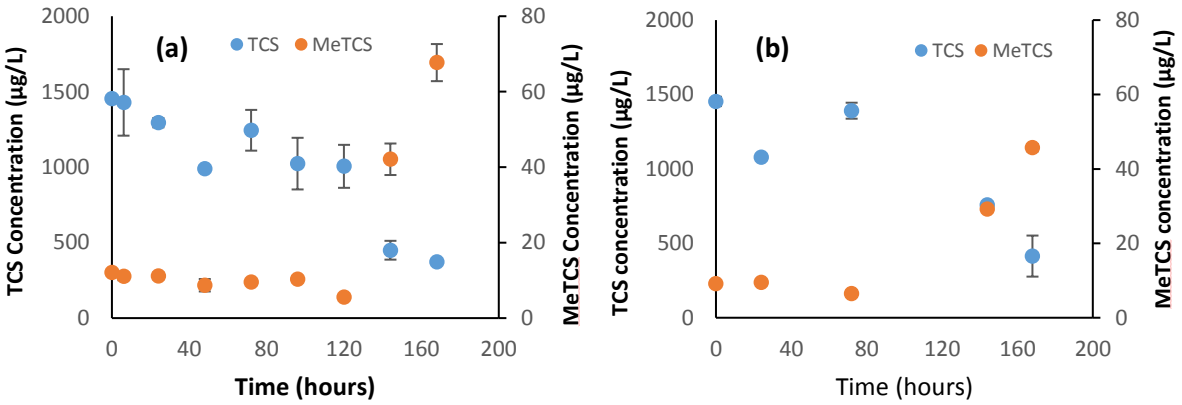


Figure 5-8 Disappearance of triclosan and formation of methyl triclosan under aerobic conditions over time (a) in reactor, (b) in inactivated control

However, only 4.8 % of triclosan was biotransformed to methyl triclosan which suggests the formation of other major triclosan transformation products. Phenol, catechol and 2, 4-dichlorophenol have been identified as the major bio-transformation products of triclosan under aerobic conditions with pure bacteria strains isolated from activated sludge (Veetil *et al.*, 2012). The observed methylation of triclosan in this study (4.8 %) was higher than the 1 % reported by (Chen *et al.*, 2011) but lower than the 42 % reported by (Armstrong *et al.*, 2018).

Surprisingly, results from the inactivated control (autoclaved sludge) showed losses after 168 hours. This was either due to incomplete inactivation of the inocula during the autoclaving process described by Helbing *et al*. (Helbling *et al.*, 2012) or problems with the autoclave. It is possible that some bacteria (or their spores) were not killed by the double autoclaving and may have regrown at the incubation temperature (25 – 29.5 ^{o}C), which differed largely from the incubation temperature of 20 ^{o}C used by Helbling and in our previous work (Section 4.2.3). The methylation of triclosan observed in the inhibition control (Figure 5-8) indicated incomplete inactivation of the sludge, since such a transformation is unlikely to occur without a biological catalyst. Therefore, associated reductions of triclosan in this control may be attributed to biodegradation, not abiotic losses.

5.3.3.1.2 Biotransformation of triclosan with WSP (facultative) inocula under aerobic conditions

Degradation of triclosan under aerobic condition using facultative inocula obtained from a WSP (WWTP C) was observed. The concentration of triclosan decreased by 63 % and 92 % under light and dark conditions respectively over the duration of the experiment (15 days) (Figure 5-10; Figure 5-9).

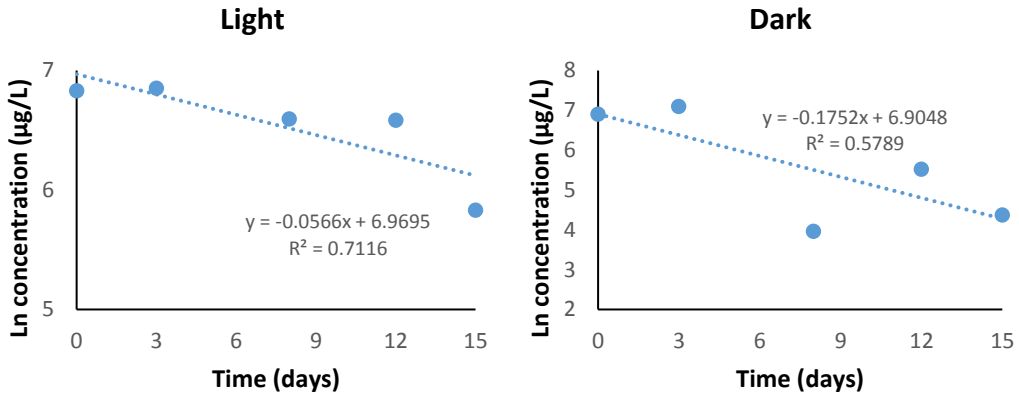


Figure 5-9 Degradation of triclosan with facultative inocula under aerobic conditions

The biodegradation of triclosan was assumed to follow first order kinetics under the light and dark conditions with a half-life of 12 days and 4 days respectively (Table 5-15; Figure 5-9), although a scatter around first order kinetics and distinct multiple phases were observed Triclosan degraded about three times faster in the dark condition with a rate constant of 0.1752 h^{-1} compared to 0.0566 h^{-1} in light conditions. Photodegradation has been reported to play a role in triclosan elimination from the environment due to its degradation when irradiated with UV light and sunlight (Chen *et al.*, 2008; Buth *et al.*, 2010; Tamura and Yamamoto, 2012). A study reported 63 % photodegradation of triclosan (at a first order rate of 0.087 d^{-1}) and formation of 2,8-Dichlorodibenzo-*p*-dioxin (2,8-DCDD) in fresh water after irradiation with white light (fluorescent lamp), and no degradation under dark conditions (Aranami and Readman, 2007). The reverse was the case in our study with facultative inocula where triclosan degraded quicker under dark conditions. This might be due to competition between the increasing number of autotrophic bacteria and heterotrophs for nutrients under light conditions leading to less degradation of triclosan. On the other hand, the death of autotrophs and subsequent release of nutrients might have favoured the cometabolic degradation of triclosan by the heterotrophs. Anaerobic conditions were not maintained in these experiments (this was a facultative pond inoculum), and the co-existence of aerobic and anaerobic conditions might have different effect on the system.

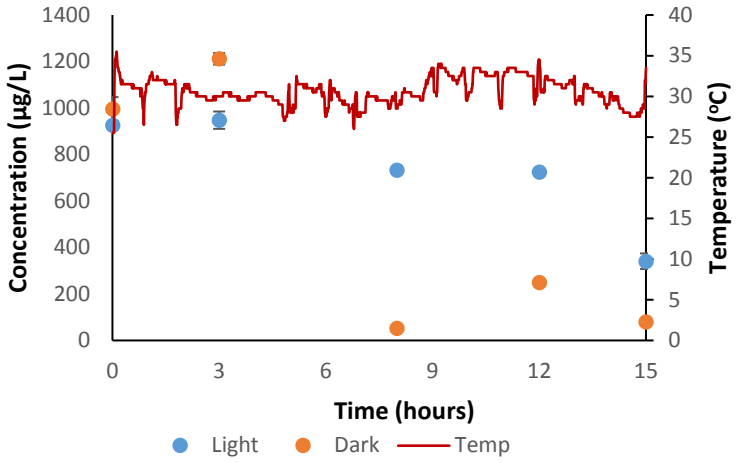


Figure 5-10 Degradation of triclosan with WSP facultative inocula under light and dark aerobic conditions with real time temperature variations

Similar to the aerobic experiment with activated sludge, the inactivated control (autoclaved inocula) also showed disappearance of triclosan, most likely as a result of higher incubation temperature (26 – 34.5 ^{o}C) used in this experiment compared to the 20 ^{o}C employed in most other studies that have inactivated their control biomass by autoclaving or failure of the autoclave. However, the uncontrolled temperature approach has the benefit of mimicking real-life temperature variations in these systems. The observed triclosan degradation in this experiment suggests that biodegradation played an important role in triclosan removal in WWTP C.

Table 5-15 First order degradation rate constant of triclosan with facultative inocula under light and dark aerobic conditions.

	Disappearance of triclosan					
	C_s (µg/L)	C_f (µg/L)	k (d^{-1})	S_k	R^2	$t_{1/2}$ (d)
Light	925	341	0.0566	0.0863	0.7116	12.2
Dark	995	80	0.1752	0.0208	0.5789	4.0

C_s and C_f represents starting and final concentration respectively; k represents the degradation rate; $t_{1/2}$ represents half-live; S_k represents standard error of the rate constant, and $t_{1/2}$ represents the half-life.

5.3.3.1.3 Biotransformation of triclosan with UASB inocula under anaerobic conditions

After 15 days of inoculation, only a slight reduction of triclosan was observed under anaerobic condition (Figure 5-11). The measured concentration of triclosan at the start of the experiment of 3397 ± 790 µg/L remained at a similar level after 15 days (2945 ± 45 µg/L). Also, methylation of triclosan was not observed in these anaerobic batch tests as the concentration of methyl triclosan remained unchanged throughout the experiment (result not shown). Chen *et al*. also reported no significant biodegradation of triclosan under anaerobic conditions with mixed culture inocula (Chen *et al.*, 2011). However, 60 – 90 % degradation of triclosan under anaerobic conditions by pure bacteria strains inoculated from anaerobic sludge has been reported (Veetil *et al.*, 2012). The lack of triclosan biodegradation under anaerobic condition indicates that the removal achieved by the UASB reactor in WWTP B (where the inocula was sourced) was solely due to sorption.

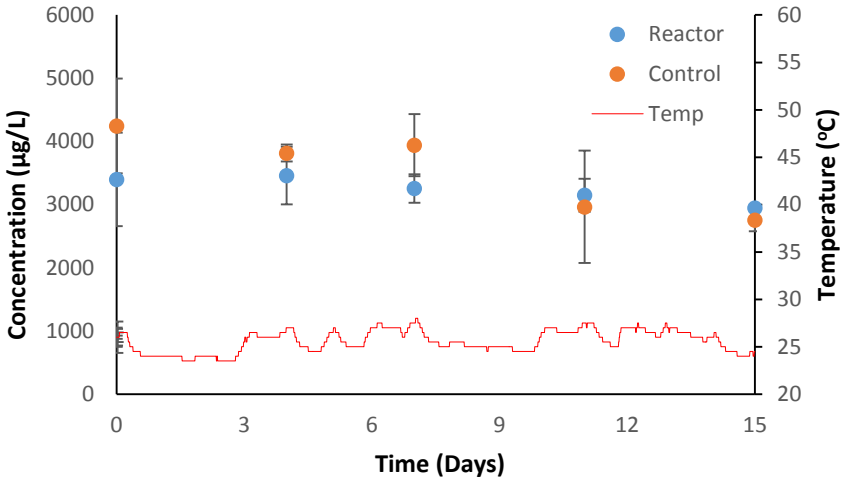


Figure 5-11 Concentration of triclosan in anaerobic batch tests inoculated with UASB sludge. Control represents autoclaved inocula

5.3.3.2 Biodegradation of PAHs under different redox conditions and inocula

5.3.3.2.1 Biotransformation of PAHs with activated sludge inocula under aerobic conditions

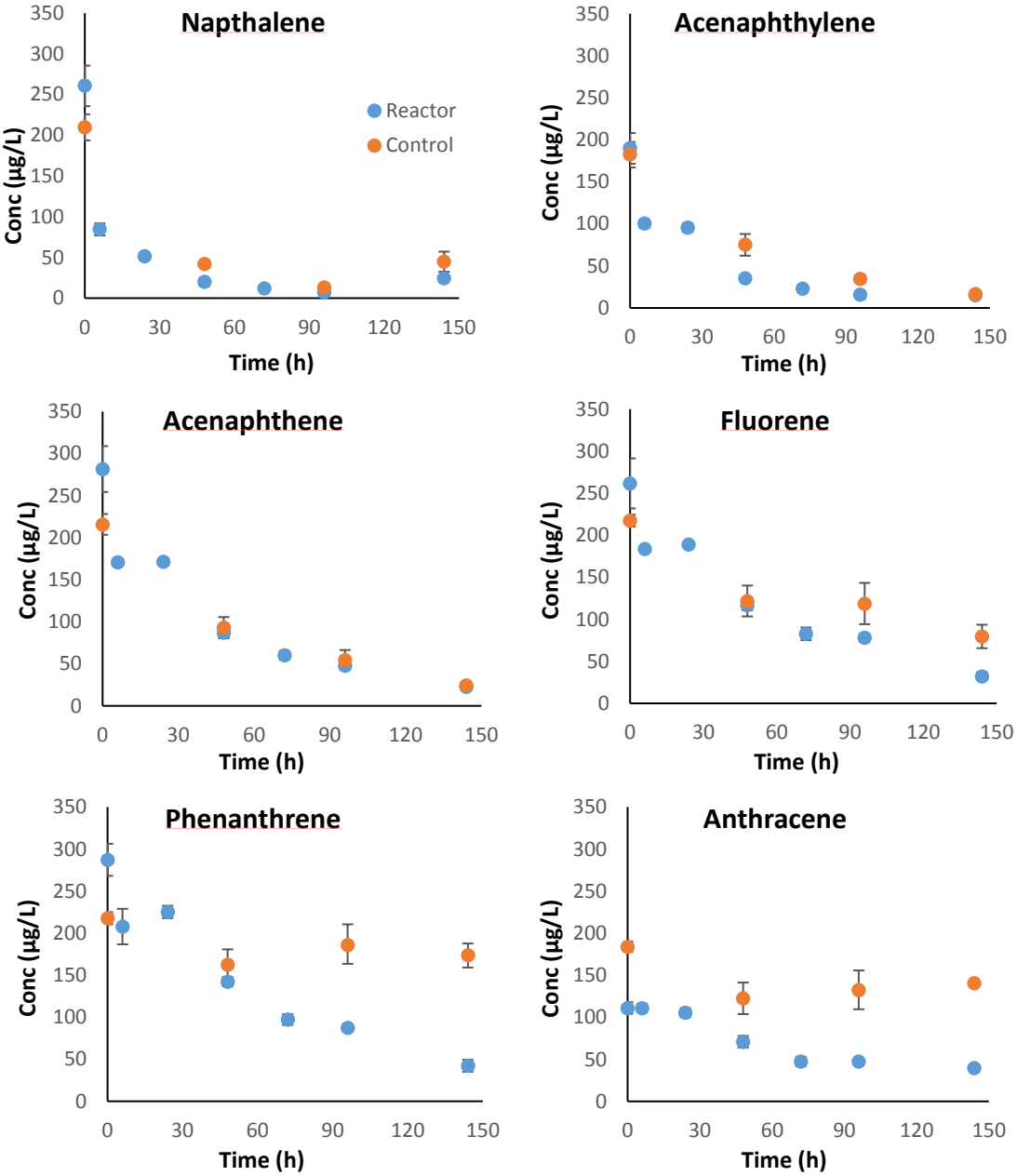


Figure 5-12 Degradation of low molecular weight PAHs under aerobic conditions with activated sludge inocula from WWTP A. Error bars represents standard deviation of triplicate measurements

Reduction of low molecular weight (LMW) PAHs ranged from 64 % for anthracene and 97 % for naphthalene after 144 hours (Figure 5-12). By comparison, reduction of middle molecular weight (MMW) PAHs ranged from 57 % for chrysene to 61 % for fluoranthene (*Figure 5-13*), while high molecular weight PAHs ranged from 56 % for benzo(b)fluoranthene to 21 % for dibenz(a,h)anthracene (Figure 5-14). The inactivated control (autoclaved activated sludge) showed reduction of some LMW PAHs- suggesting that volatilization contributed to the reduction of LMW PAHs (naphthalene, acenaphthylene, acenaphthene and fluorene) as reported in previous experiment (Section 4.5.4). Volatilization tendency for these four chemicals were estimated to be between 0.5 – 2 % at 25 ^{o}C and atmospheric pressure (see calculation in Appendix C- 8.3), but aeration during activated sludge treatment has been reported to intensify volatilization rates (Luo *et al.*, 2014). Lighter PAHs tend to volatilise as a result of their relatively lower melting point and higher water solubility compared to heavier PAHs (Trably *et al.*, 2005).

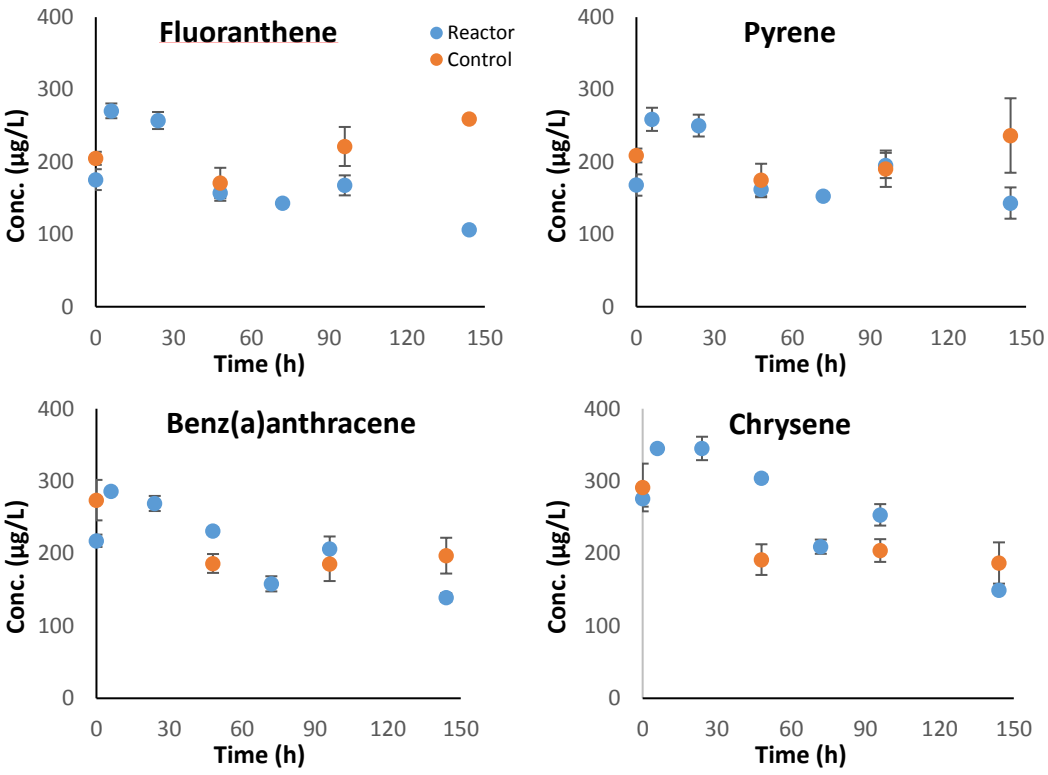


Figure 5-13 Degradation of middle molecular weight PAHs under aerobic conditions with activated sludge inocula from WWTP A. Error bars represent standard deviation of triplicate measurements

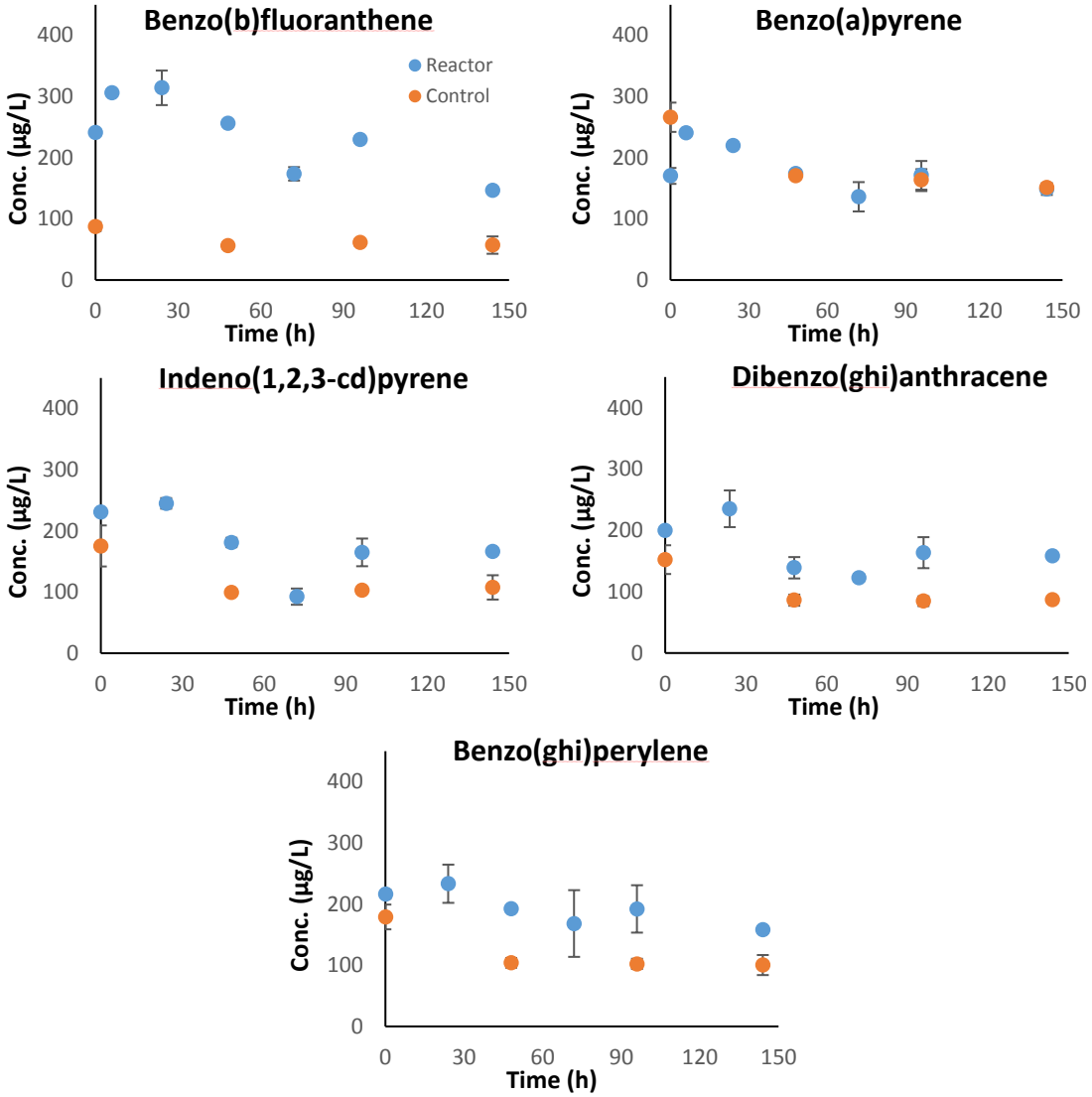


Figure 5-14 Degradation of high molecular weight PAHs under aerobic conditions with activated sludge inocula from WWTP A. Error bars represent standard deviation of triplicate measurements

The half-lives and rate constants for the degradation of LMW PAHs and MMW/HMW PAHs were calculated from the data between 0 h - 144 h (0 h – 96 h for naphthalene), and 24 h to 144 h respectively. The data at 0 h was omitted in these calculations for MMW and HMW PAHs due to the observed adaptation phase (where the concentration of the PAHs increased probably due to equilibration and solubilisation) in the growth curve. Furthermore, data at 6 hours was omitted for HMW PAHs as there appeared to be issues associated with measurement of these chemicals at this point. Regression analysis showed that these omitted data points gave large residual values and its inclusion lead to poorer R^2 values.

Table 5-16 Degradation rate constants (k) of degraded PAHs under aerobic conditions with activated sludge inocula.

Compound	C_s (µg/L)	C_f (µg/L)	k (h^{-1})	S_k	R^2	$t_{1/2}$ (h)
Naphthalene	261	7	0.0340	0.0049	0.9238	20.4
Acenaphthylene	190	15	0.0180	0.0033	0.8585	38.5
Acenaphthene	283	23	0.0165	0.0013	0.9684	42.0
Fluorene	261	32	0.0134	0.0011	0.9680	51.7
Phenanthrene	287	42	0.0126	0.0010	0.9721	55.0
Anthracene	111	40	0.0082	0.0012	0.8984	84.5
Fluoranthene	271	106	0.0064	0.0017	0.8229	108.3
Pyrene	259	143	0.0039	0.0016	0.6054	177.7
Benz(a)anthracene	286	139	0.0051	0.0013	0.9560	135.9
Chrysene	345	149	0.0061	0.0013	0.8693	113.6
Benzo(b)fluoranthene	306	146	0.0055	0.0014	0.7968	126.0
Benzo(a)pyrene	240	149	0.0034	0.0013	0.6015	203.8
Indeno(1,2,3-cd)pyrene	231	166	0.0032	0.0029	0.2227	216.6
Dibenz(a,h)anthracene	200	158	0.0022	0.0020	0.2334	315.0
Benzo(ghi)perylene	216	158	0.0024	0.0008	0.7084	288.8

C_s and C_f represents starting and final concentration respectively; k represents the degradation rate; $t_{1/2}$ represents half-live; S_k represents standard error of the rate constant, and $t_{1/2}$ represents the half-life.

The biodegradation of PAHs was assumed to follow first order kinetics and their half-lives ranged from 20 – 347 hours (Table 5-16), although a scatter around first order kinetics and distinct multiple phases were observed. The half-lives and first order rate constants of LMW PAHs ranged from 20 – 85 hours (naphthalene to anthracene) and 0.0340 – 0.0082 h^{-1} (naphthalene to anthracene) respectively. Since volatilization was observed to contribute the removal of some LMW PAHs, their first order volatilization rates were estimated (Figure 8-17, Section 8.4) and they ranged from 0.0291 h^{-1} for naphthalene, 0.0167 h^{-1} for acenaphthylene, 0.0147 h^{-1} for acenaphthene and 0.0063 h^{-1} for fluorene. .In comparison, slower rates and longer half-lives were observed for MMW PAHs with half-lives and rate constants ranging from 110 – 182 h (fluoranthene- pyrene) and 0.0063 – 0.0038 h^{-1} (fluoranthene- pyrene) respectively (Table 5-16). HMW PAHs were the slowest to degrade with half-lives and rate constants ranging from 131 – 347 hours and 0.0053 – 0.0020 h^{-1}. This further suggests that degradation rates of PAHs increases from light to heavier PAH under aerobic conditions, and agrees with findings by several reports in literature (Trably *et al.*, 2005; Ghosal *et al.*, 2016).

5.3.3.2.2 Biotransformation of PAHs with WSP (facultative) inocula under aerobic conditions

Degradation of 15 PAHs under aerobic conditions with facultative inocula was observed in the presence and absence of white light (fluorescent bulb placed 20 cm from the reactors) (Figure 5-15, Figure 5-16, Figure 5-17). The inactivated control (autoclaved sludge) failed in this experiment, so abiotic losses could not be accounted for. The reduction of LMW PAHs ranged from 80 – 98 % and 79 – 99 % (anthracene to naphthalene) under light and dark conditions respectively (Figure 5-15). In comparison, the reduction of MMW PAHs was less, ranging from 30 – 74 % and 22 – 79 % (chrysene to fluoranthene) under light and dark conditions respectively (Figure 5-16).

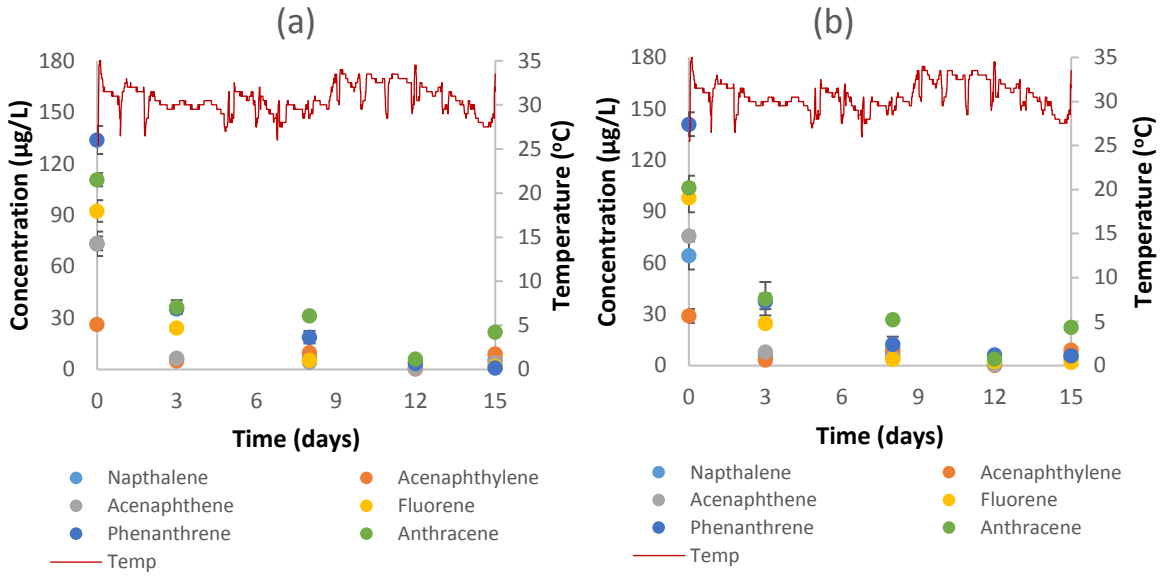


Figure 5-15 Degradation of low molecular weight PAHs under aerobic conditions with facultative pond inocula (a) Light, (b) Dark (Error bars replicate standard deviation of triplicate measurements

Unsurprisingly, the extent of degradation of HMW PAHs was lowest, ranging from 31 – 45 % and 8.2 % to 32 % (benzo(b)fluoranthene to benzo(a)pyrene) under the light and dark conditions respectively (Figure 5-17).

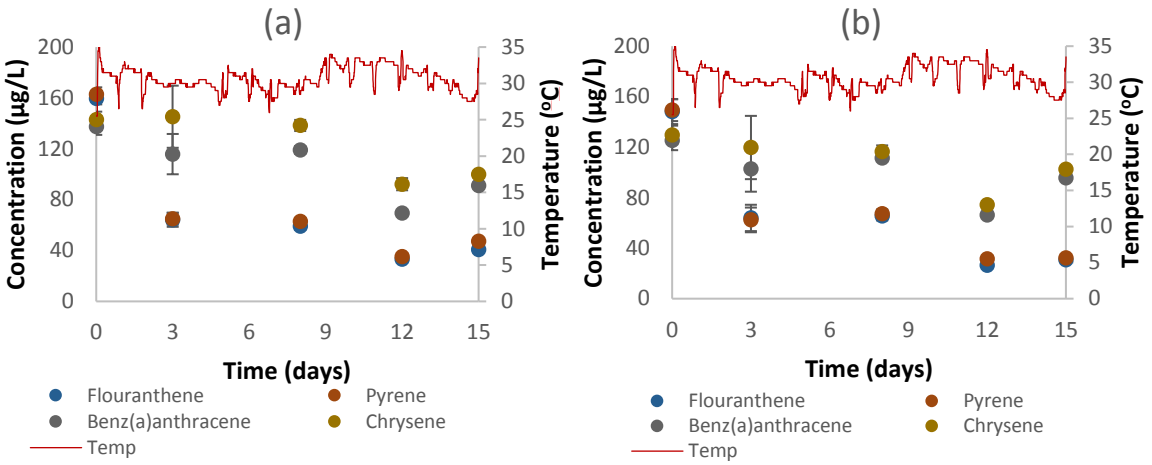


Figure 5-16 Degradation of middle molecular weight PAHs under aerobic conditions with facultative pond inocula (a) Light, (b) Dark (Error bars replicate standard deviation of triplicate measurements.

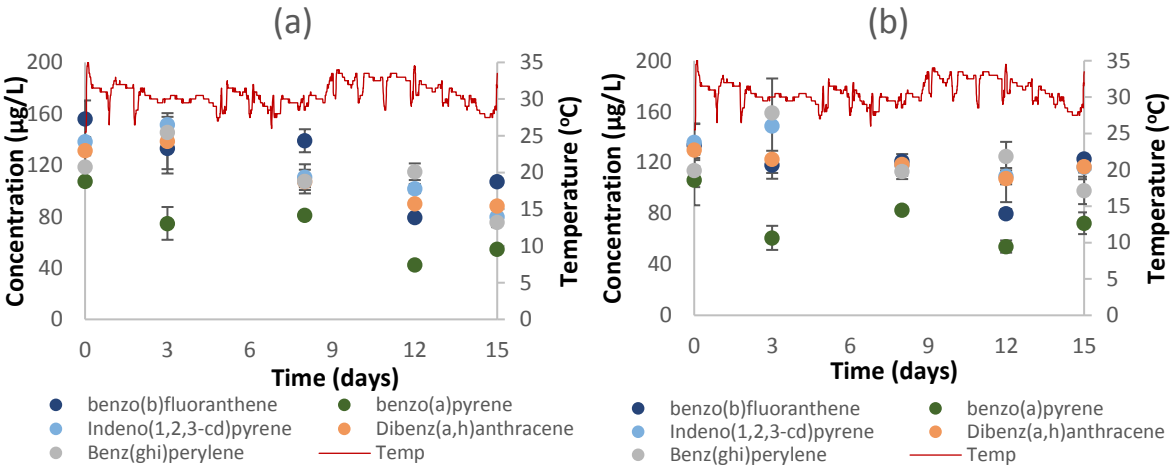


Figure 5-17 Degradation of high molecular weight PAHs under aerobic conditions with facultative pond inocula (a) Light, (b) Dark (Error bars replicate standard deviation of triplicate measurements.

The degradation of PAHs was assumed to follow first order kinetics with half-lives ranging from 2 – 34 days and 2 – 77 days under light and dark conditions (Table 5-17, Figure 5-15, Figure 5-16, Figure 5-17); although a scatter around first order kinetics and distinct multiple phases were observed. Degradation was slower with increasing molecular weight of the PAHs since rate constants were between 0.0863 to 0.3833 d^{-1}, 0.0253 – 0.1024 d^{-1} and 0.0090 to 0.0490 d^{-1} for LMW, MMW and HMW PAHs respectively (Table 5-17). Similar degradation rates and half-lives were observed for LWM PAHs under light (2 days) and dark (2 days)

conditions with similar rate constants, except for phenanthrene and anthracene, where the reaction was relatively faster in the light (half-life = 2 and 5 days respectively in light, 3 and 8 days in the dark) (Table 5-17). The degradation of MMW PAHs was faster under light (half-lives ranging from 8 – 23 days) than dark conditions (half-lives ranging from 7.7 – 27 days). Similarly, degradation of HMW PAHs was faster in the light (half-lives between 14 – 24 days), than dark conditions (half-lives between 18 – 77 days).

Photolysis has been reported as major abiotic degradation process for PAHs either via direct photooxidation (radiation absorbed directly by PAHs) or photosensitization (transformation mediated by other light absorbing substances) (Bertilsson and Widenfalk, 2002; Saeed *et al.*, 2011). This explains the observed faster degradation under light conditions. Furthermore, photolytic degradation has been reported to be more rapid in higher molecular weight PAHs due to their lower quantum yields that leads to increased photoreactivity by a better overlap of their adsorption to solar spectrum (Kochany and Maguire, 1994). This explains the extent of high molecular weight PAHs photodegradation in presence of light compared to low and middle molecular weight one in this study.

Table 5-17 Degradation rate constants (*k*) of degraded PAHs under aerobic conditions with facultative inocula in the light and dark

	Light				Dark			
Compound	***k* (d^{-1})**	**S_k**	**R^2**	**$t_{1/2}$ (d)**	***k* (d^{-1})**	**S_k**	**R^2**	**$t_{1/2}$ (d)**
Naphthalene	0.2890	0.0957	0.8202	2.4	0.3046	0.1034	0.8144	2.3
Acenaphthylene	0.3129	0.1447	0.7004	2.2	0.3337	0.1677	0.6644	2.1
Acenaphthene	0.3443	0.0848	0.8917	2.0	0.3833	0.0839	0.9125	1.8
Fluorene	0.2472	0.0323	0.9514	2.8	0.2641	0.0461	0.9164	2.6
Phenanthrene	0.2995	0.0351	0.9605	2.3	0.2111	0.0336	0.9292	3.3
Anthracene	0.1313	0.0603	0.6123	5.3	0.0904	0.0371	0.7483	7.7
Fluoranthene	0.0863	0.0265	0.7792	8.0	0.1024	0.0258	0.8402	6.8
Pyrene	0.0787	0.0286	0.7165	8.8	0.0947	0.0241	0.8378	7.3
Benz(a)anthracene	0.0347	0.0150	0.6395	20.0	0.0257	0.0169	0.4343	27.0
Chrysene	0.0305	0.0102	0.7503	22.7	0.0253	0.0143	0.5117	27.4
Benzo(b)fluoranthene	0.0330	0.0161	0.5856	21.0	0.0173	0.0165	0.2704	40.1
Benzo(a)pyrene	0.0490	0.0180	0.7123	14.1	0.0385	0.0143	0.7065	18.0
Indeno(1,2,3-cd)pyrene	0.0388	0.0081	0.6831	17.9	0.0169	0.0066	0.8851	41.0
Dibenz(a,h)anthracene	0.0322	0.0057	0.9142	21.5	0.0090	0.0038	0.6535	77.0
Benzo(ghi)perylene	0.0291	0.0145	0.5711	23.8	0.0184	0.0126	0.4155	37.7

C_s and C_f represents starting and final concentration respectively; k represents the degradation rate; $t_{1/2}$ represents half-live; S_k represents standard error of the rate constant, and $t_{1/2}$ represents the half-life.

5.3.3.2.3 Biotransformation of PAHs with UASB inocula under anaerobic conditions

Biodegradation of the 15 investigated PAHs (ranging from high – middle molecular weight PAHs) was not observed under anaerobic condition with UASB inocula in 15 days (Figure 5-18). Furthermore, the concentration of the 15 PAHs in the inhibition and abiotic control (data not shown) remained at the same level throughout the experiment. This indicates that none of the PAHs, especially the low molecular weight PAHs, were lost due to volatilization or non-biological degradation. These results suggests that removal of PAHs observed after UASB treatment in WWTP B was mostly due to adsorption onto solids and volatilization (for LMW PAHs).

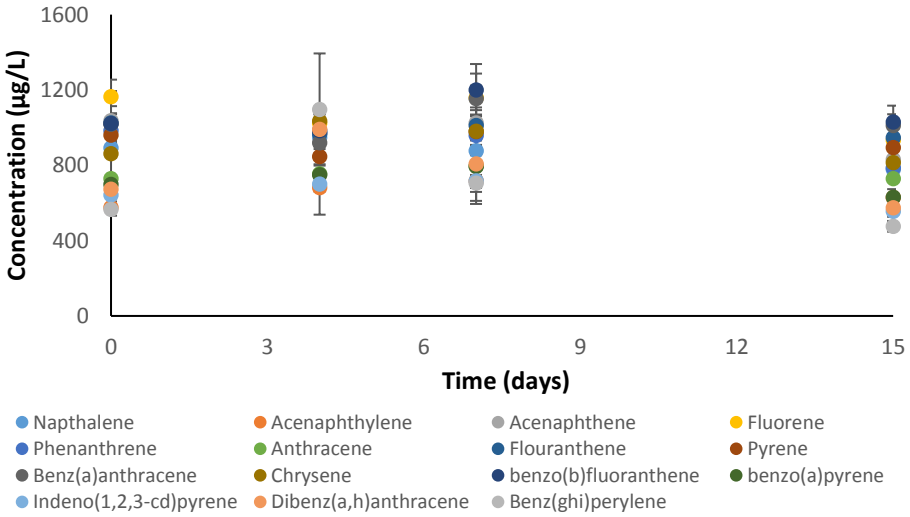


Figure 5-18 Degradation of PAHs under anaerobic conditions with UASB inocula from WWTP B

5.3.3.3 Biodegradation of estrogens under different redox conditions and inocula

5.3.3.3.1 Biotransformation of estrogens with activated sludge inocula under aerobic conditions

The reduction of all four estrogens observed in the experiment was solely due to biodegradation as no sign of abiotic losses was indicated by the inactivated control (autoclaved sludge) (Appendix B- 8.2). The concentration of E1, E2, E3 decreased by 99 % in 72 hours in the batch tests; with the most reduction (96 – 99 %) occurring within 24 hours (Figure 5-19). EE2 was also reduced by 88 % after 72 hours (Figure 5-19). Rapid transformation and high removal rate of E3, E2, E1 and EE2 has been reported to occur under nitrifying conditions with activated sludge and ammonia oxidizing bacteria (Haiyan *et al.*, 2007; Dytczak *et al.*, 2008; Gaulke *et al.*, 2008). In our study, E3 and E1 were the least adsorbed estrogen as 96 – 97 % and 92 – 96 % respectively was present in the aqueous phase, compared to EE2 (25 – 47 %) and E2 (8.2 – 8.5 %). This might be due to their water solubility and Log K_{ow} (2.47 for E3, 3.13 for E1, 3.67 for EE2 and 4.10 for E2) (Liu *et al.*, 2009) and implies that the relatively highly hydrophobic EE2 and E2 possess higher tendency of adsorption when compared to E3 and E1, hence, their removal in treatment plants can be partly due to adsorption (Urase and Kikuta, 2005; Wang *et al.*, 2013a).

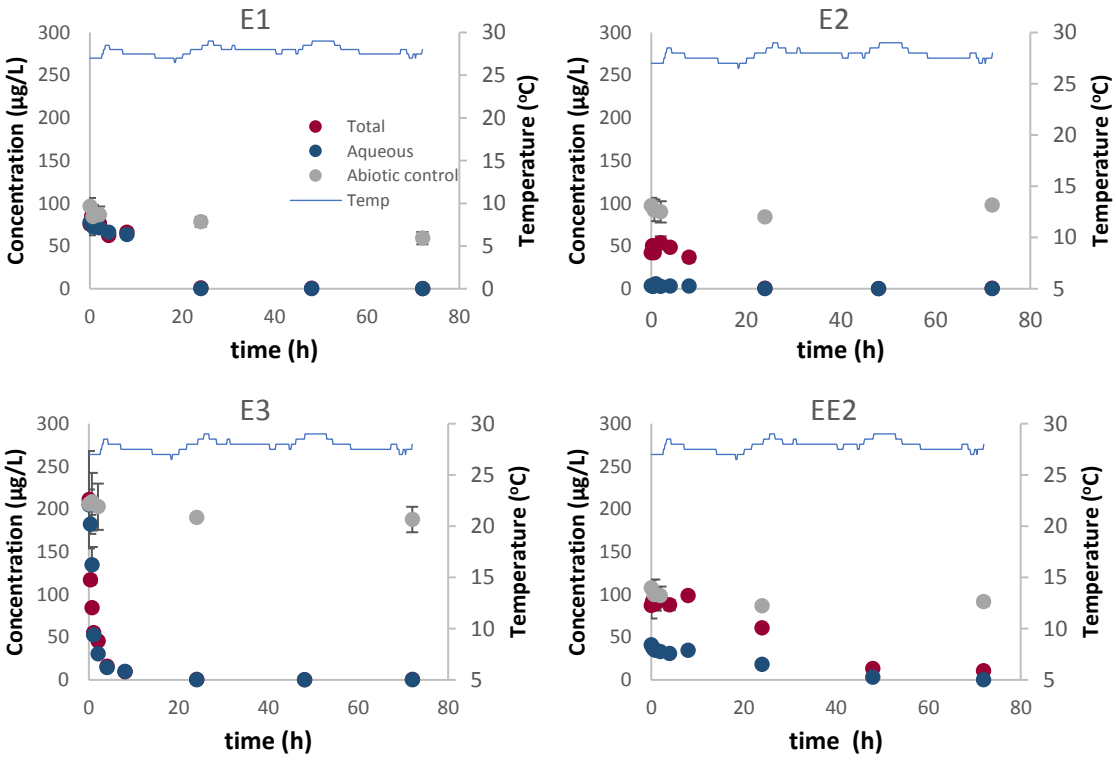


Figure 5-19 Degradation of estrogens under aerobic conditions with activated sludge inocula from WWTP A. Error bars represents standard deviation of triplicate measurements

Table 5-18 Degradation rate constants (*k*) of degraded estrogens under aerobic conditions with activated sludge inocula.

Compound	Sludge conc. (g/L)	Temp. (°C)	C_s (µg/L)	*k* (h^{-1})	S_k	$t_{1/2}$ (h)	References
E1	4.3	20	76.0*	0.1261	0.0163	5.5	This study
	2.7	30	1000	0.0560	-	12.4	Shi *et al*., 2004
	1.0	18	0.1	0.3500	-	2.0	Petrie *et al*., 2014
E2	4.3	20	42.1*	0.1496	0.0145	4.6	This study
	2.7	30	1000	1.3000	-	0.5	Petrie *et al*., 2014
	1.0	18	0.1	0.2700	-	2.6	Shi *et al*., 2004
E3	4.3	20	211*	0.1426	0.0217	4.9	This study
	2.7	30	1000	0.0300	-	23.1	Petrie *et al*., 2014
	1.0	18	0.1	0.3300	-	2.1	Shi *et al*., 2004
EE2	4.3	20	87.1*	0.0331	0.0030	20.9	This study
	2.7	30	1000	0.0350	-	19.8	Petrie *et al*., 2014

*C_s represents spiked concentration;*measured concentration reported (spiked at 100 µg/L; k represents the degradation rate;* S_k represents standard error of the rate constant, *$t_{1/2}$ represents half-live,*

The biotransformation of the all four estrogens was suggested to follow first order kinetics with estimated half-lives of 5.5 h, 4.6 h, 4.9 h, and 20.9 h for E1, E2, E3 and EE2 respectively (Figure 5-19, Table 5-18); although a scatter around first order kinetics and distinct multiple phases were observed Furthermore, the rate constants estimated for E2 (0.1496 h^{-1}) and E3 (0.1426 h^{-1}), were not significantly different from each other (p-value > 0.05, one-way ANOVA), while E1 (0.1261 h^{-1}), and EE2 (0.0331 h^{-1}) were significantly different (p-value < 0.05, one-way ANOVA). Reported degradation rates of estrogens varies greatly in the literature because the biodegradation assays are conducted under different temperatures, inocula concentration and spiked concentrations (Liu *et al.*, 2015) (Table 5-18). The degradability of the estrogens in our study is E3/E2 > E1 > EE2. This observation is in agreement with previous studies (Petrie *et al.*, 2014; Liu *et al.*, 2015). However, Shi *et al*., reported that E3 was the most resistant to aerobic degradation (Shi *et al.*, 2004) (Table 5-19).

5.3.3.3.2 Biotransformation of estrogens with WSP (facultative) inocula under aerobic conditions

The reduction of all four estrogens was observed in the aerobic batch tests under light and dark conditions (Figure 5-20). Although, no other studies have used facultative inocula in their experiments, the degradation trend observed was similar to those reported with activated sludge inocula (Dytczak *et al.*, 2008). About 99.5 % of E1, E2 and E3 were degraded after 15 days under the light and dark conditions (Figure 5-20). Also, more EE2 was degraded under light conditions (99.5%), than in the dark (93.5%) after 15 days (Figure 5-20). Most of the losses of E1, E2 and E3 (>95 %) occurred within 8 days and 12 days under dark and light conditions respectively. However, degradation of EE2 was slower, and was mostly removed after 12 days.

The degradation of all four estrogens under light and dark incubation conditions was suggested to follow first order kinetics (Figure 5-20), although a scatter around first order kinetics and distinct multiple phases were observed. The reaction kinetics was similar for E1 and E3 under both light and dark conditions with estimated rate half-lives of 1.4 days (Table 5-19). Meanwhile the degradation reaction kinetics was faster under light conditions for E2 and EE2. The rate constant of E2 (half-life of 1.6 days) was 0.4368 d^{-1} under light conditions, compared to 0.3888 d^{-1} (half-life of 1.8 days) under dark conditions. Also, the degradation rate almost doubled in the light (k = 0.3024 d^{-1}, half-life = 2.3 days) for EE2 compared to the dark conditions (k = 0.1632 d^{-1}, half-life = 4.2 days) (Table 5-19).

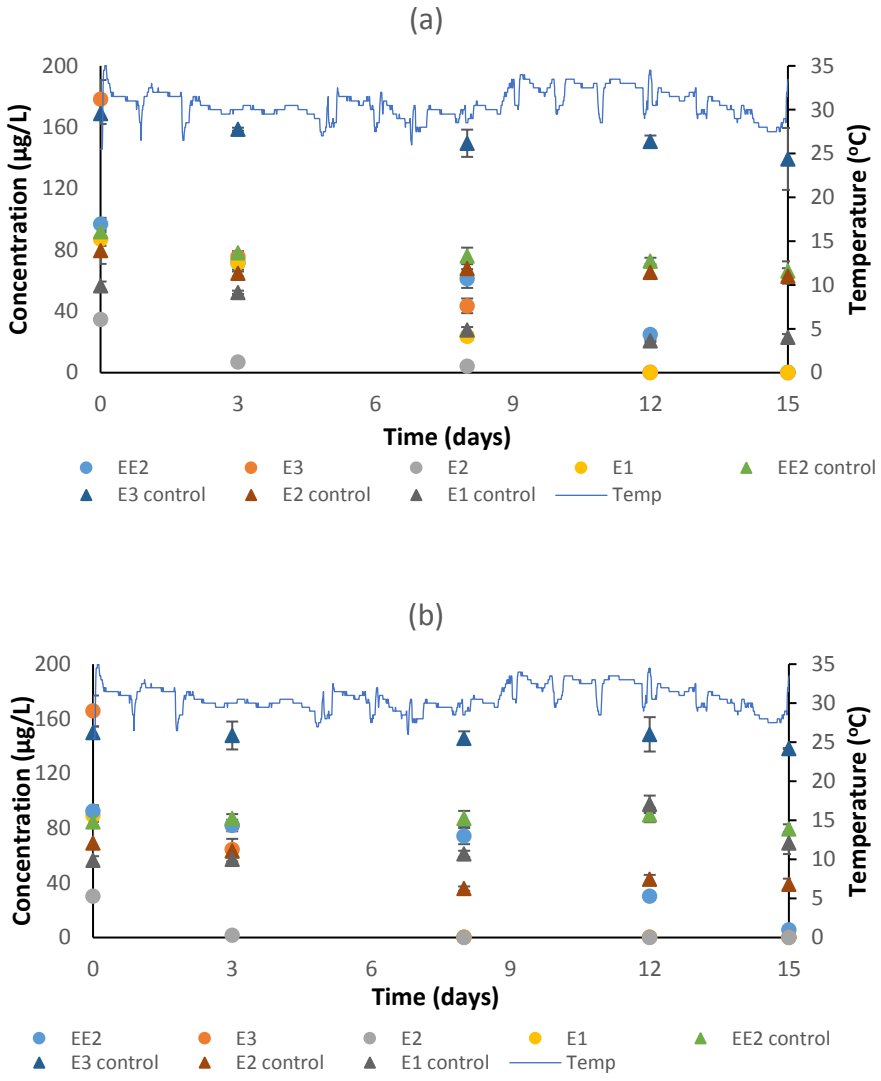


Figure 5-20 Degradation of estrogens under aerobic conditions with facultative inocula from WWTP C under (a) light and (b) dark conditions. Error bars represents standard deviation of triplicate measurements

This enhanced degradation E2 and EE2 under light conditions was due to photolysis in which the compounds might have degraded directly by absorption of photons or through photosensitization (Zhang and Zhou, 2005; Sornalingam *et al.*, 2016). A review of several publications on the photodegradation of estrogens published recently by Sornalingam *et al.* reports photodegradation of estrogens under different light sources (sunlight, visible light, UV light- with UV light most effective) and water matrixes (Sornalingam *et al.*, 2016). It was established that the rate of photodegradation of estrogens is influenced by light source and intensity, and solution matrix (Sornalingam *et al.*, 2016). In fact, the same light source was

reported to have different effects on individual estrogens, as E1, E2 and EE2 were removed at a similar rate under catalysis, but removal followed the order EE2 > E1 > E2 under UVA light (Coleman *et al.*, 2004). This might explain the observed photodegradation of E2 and EE2, and not for E1 and E3 as individual chemicals can behave differently sometimes.

Table 5-19 Degradation rate constants (*k*) of estrogens under aerobic conditions with facultative inocula in the light and dark

	Light				Dark			
Compound	*k* (d^{-1})	S_k	R^2	$t_{1/2}$ (d)	*k* (d^{-1})	S_k	R^2	$t_{1/2}$ (d)
E1	0.4920	0.1246	0.8384	1.4	0.5064	0.1661	0.7564	1.4
E2	0.4368	0.0830	0.9024	1.6	0.3888	0.0884	0.8663	1.8
E3	0.4872	0.1080	0.8713	1.4	0.5016	0.1318	0.8279	1.4
EE2	0.3024	0.1304	0.6433	2.3	0.1632	0.0549	0.7491	4.2

k represents the degradation rate; S_k represents standard error of the rate constant, $t_{1/2}$ *represents half-live*

5.3.3.3.3 Biotransformation of estrogens with UASB inocula under anaerobic conditions

After 15 days of incubation, no reduction of E1, E2, E3 and EE2 was observed in the anaerobic batch tests as the concentration levels remained the same (Figure 5-21). The levels of E1, E2, E3 and EE2 also stayed the same for 15 days in the inhibition control, indicating no abiotic losses or non-biological degradation (result not shown). This observation is in contrast with previous studies that have reported anaerobic degradation of estrogens, especially E1 and E2 (Andersen *et al.*, 2004; Zhang *et al.*, 2015). However, several other studies reported the resistance of EE2 to degradation under anaerobic conditions (Joss *et al.*, 2004; Czajka and Londry, 2006). Furthermore, anaerobic degradation of E3 has not been reported in any study to my knowledge, even though it is the most easily degraded estrogen under aerobic conditions. Either activated sludge or lake sediment was used as inocula in the previous studies that reported anaerobic degradation of estrogens. UASB sludge was used as inocula in this study, and this might explain the lack of degradation when compared to past studies.

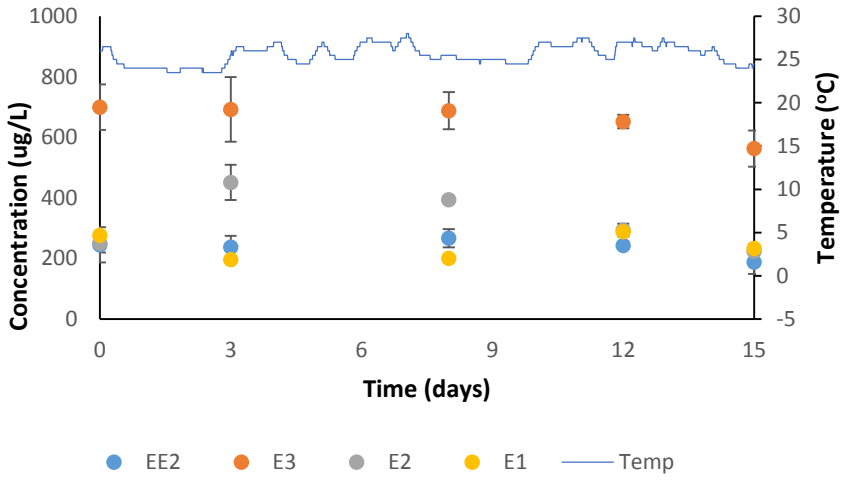


Figure 5-21 Degradation of estrogens under anaerobic conditions with UASB inocula from WWTP B

5.3.3.4 Biodegradation of PBDEs under different redox conditions and inocula

5.3.3.4.1 Biotransformation of PBDEs with WSP (facultative) inocula under aerobic conditions

Reduction in the concentration of individual PBDE congeners ranged from 40 – 63 % and 42 – 63 % under light and dark conditions respectively (Figure 5-22). Furthermore, degradation of the most abundant congener BDE 209 was 40 % and 57 % under light and dark conditions respectively. Similar results were reported by Stiborova *et al*. where degradation of BDE 28 to BDE 209 was between 62 – 78% after 11 months of studies in aerobic reactors inoculated with sewage sludge and placed in the dark (Stiborova *et al.*, 2015).

The degradation of the PBDE congeners was assumed to follow first order kinetics with half-lives ranging from 10.4 – 24.3 days and 10 – 11.5 days under light and dark conditions respectively (Table 5-20, Figure 5-22); although a scatter around first order kinetics and distinct multiple phases were observed. The rate constants of individual PBDE congers under the light and dark conditions were significantly different from each other ($p < 0.05$, one-way ANOVA). The degradation rates were relatively faster in the dark conditions with rate constants between 0.0599 – 0.0692 d^{-1} and 0.0285 – 0.0666 d^{-1} in dark and light conditions respectively. This was true for all the congeners except BDE 28, where the rate was faster under white light. This is surprising as photodegradation has been suggested as an important abiotic biotransformation process for PBDEs in the environment, with an observed debromination of heavily brominated congeners (such as BDE 209, BDE 183) to less

brominated ones (such as BDE 28, BDE 47) (Ahn *et al.*, 2006; Davis and Stapleton, 2009; Pan *et al.*, 2016).

A recent review on photodegrdation of PBDEs by Pan *et al*. showed reductive debromination of PBDEs in different aquatic systems with a range of irradiation sources (xenon or mercury or UV lamps, sunlight (Pan *et al.*, 2016). They also suggested that chemical species such as humic substances and ions (halides and metals) present in the aquatic systems strongly influences photochemical transformations. Furthermore, the source and intensity of light affects photolytic degradation of pollutants (Sornalingam *et al.*, 2016). In this study, the chemical species of the system and the light source (white fluorescent light) might not have facilitated photodegradation, and losses of PBDEs observed was biodegradation by the present microflora. Furthermore, the increased reaction kinetics in the dark might have been due to the death of autotrophs over time leading to more available nutrients for the heterotrophs to co-metabolically degrade the PBDEs.

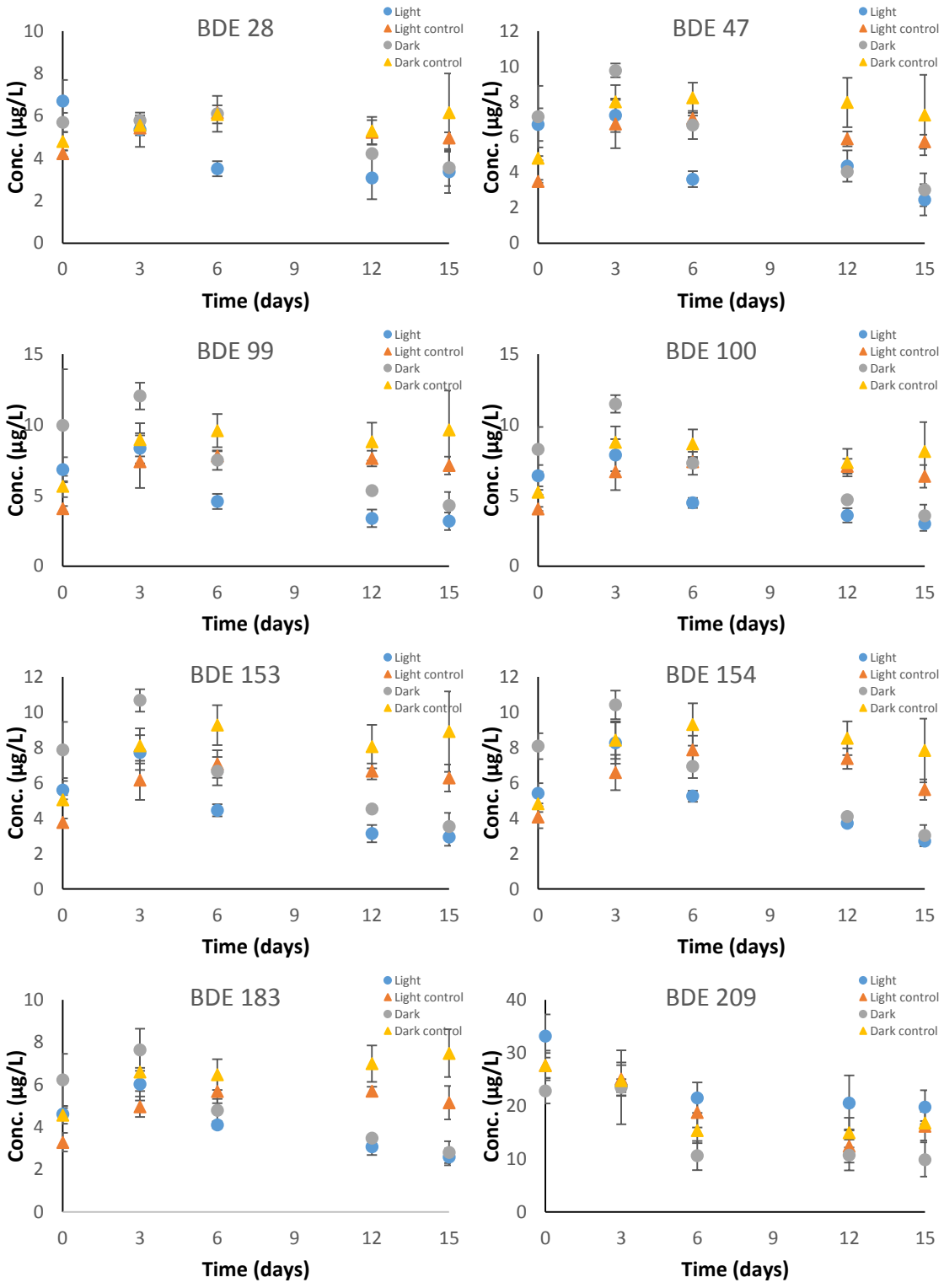


Figure 5-22 Degradation of PBDEs under aerobic conditions with facultative inocula from WWTP C. Error bar represents standard deviation of triplicate measurements

Table 5-20 Degradation rate constants (*k*) of PBDEs under aerobic conditions with facultative inocula in the light and dark

	Light				Dark			
Compound	k (d^{-1})	S_k	R^2	$t_{1/2}$ (d)	k (d^{-1})	S_k	R^2	$t_{1/2}$ (d)
BDE 28	0.0666	0.0149	0.8829	10.4	0.0599	0.0097	0.9966	11.6
BDE 47	0.0612	0.0217	0.7255	11.3	0.0692	0.0179	0.8323	10.0
BDE 99	0.0626	0.0157	0.8411	11.1	0.0648	0.0131	0.8911	10.7
BDE 100	0.0590	0.0146	0.8454	11.7	0.0677	0.0179	0.8269	10.2
BDE 153	0.0575	0.0175	0.7832	12.1	0.0645	0.0166	0.8336	10.7
BDE 154	0.0578	0.0203	0.7294	12.0	0.0670	0.0165	0.7584	10.3
BDE 183	0.0479	0.0137	0.8027	14.5	0.0616	0.0133	0.8773	11.3
BDE 209	0.0285	0.0101	0.7252	24.3	0.0604	0.0211	0.7320	11.5

k represents the degradation rate; S_k represents standard error of the rate constant*; $t_{1/2}$ represents half-live*

5.3.3.4.2 Biotransformation of PBDEs with UASB inocula under anaerobic conditions

After 15 days of incubation, no reduction of the eight PBDEs investigated was observed under anaerobic conditions with UASB inocula (Figure 5-23). This was surprising as there have been several reports of debromination of PBDEs by dehalogenating bacteria under anaerobic conditions (Gerecke *et al.*, 2005; He *et al.*, 2006; Xia, 2013). Yen *et al*. reported less than 20 % anaerobic degradation of BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154 after 70 days with mixed culture inocula sourced from river sediment (Yen *et al.*, 2009). Gereke et al. also reported 30 % debromination and degradation of BDE 209 within 238 days with digested sewage sludge under anaerobic conditions (Gerecke *et al.*, 2005). Both studies reported poor degradation of PBDEs after several months. This explains why degradation of PBDEs was not observed in this study, as the incubation period was comparatively low (15 days). Consequently, these compounds will not degrade during UASB treatment of wastewater in real WWTPs. This explains the poor removal of PBDEs in the aqueous phase after UASB treatment in WWTP B.

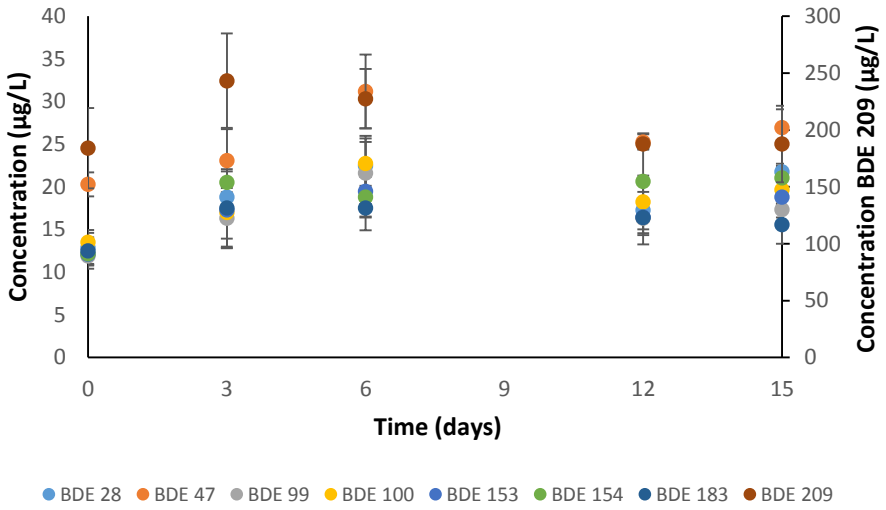


Figure 5-23 Degradation of PBDEs under anaerobic conditions with UASB inocula from WWTP B

5.3.3.5 Comparing the degradation rates of the different classes of chemicals under aerobic condition

The degradation rates of the different chemicals were compared to understand the effect of their chemical structure and functional groups on their degradability Among the different classes of chemicals investigated, estrogens degraded more rapidly with their average reaction rate 7, 16, 21 and 34 times higher than those for low molecular weight (LWM) PAHs, triclosan, middle molecular weight (MMW) PAHs and high molecular weight (HMW) PAHs respectively (Table 5-21). LMW PAHs degraded two times faster than triclosan on average. Degradation of triclosan was slightly faster (1.3 times) than MMW PAHs but was 2 times faster than HMW PAHs (Table 5-21). The difference in chemical; structure and functional groups among the chemical classes influenced their biodegradation abilities- as previously explained in Section 4.5.6.

Table 5-21 Comparing the degradation rates of different classes of chemicals under aerobic conditions with activated sludge inocula

Compound	Class of chemical	First order rates (h^{-1})	$t_{1/2}$ (h)	Class average rate (h^{-1})
Triclosan	PCP	0.0072	96.3	0.0072
Naphthalene	LMW PAHs	0.0340	20.4	0.0171
Acenaphthylene	LMW PAHs	0.0180	38.5	
Acenaphthene	LMW PAHs	0.0165	42.0	
Fluorene	LMW PAHs	0.0134	51.7	
Phenanthrene	LMW PAHs	0.0126	55.0	
Anthracene	LMW PAHs	0.0082	84.5	
Fluoranthene	MMW PAHs	0.0063	108.3	0.0054
Pyrene	MMW PAHs	0.0038	177.7	
Benz(a)anthracene	MMW PAHs	0.0050	135.9	
Chrysene	MMW PAHs	0.0060	113.6	
Benzo(b)fluoranthene	HMW PAHs	0.0053	126.0	0.0033
Benzo(a)pyrene	HMW PAHs	0.0032	203.8	
Indeno(1,2,3-cd)pyrene	HMW PAHs	0.0028	216.6	
Dibenz(a,h)anthracene	HMW PAHs	0.0020	315.0	
Benzo(g,h,i)perylene	HMW PAHs	0.0023	288.8	
EE2	Steroidal hormones	0.1261	20.9	0.1129
E1	Steroidal hormones	0.1496	5.5	
E2	Steroidal hormones	0.1426	4.6	
E3	Steroidal hormones	0.0331	4.9	

**PCP represents personal care product; * LMW, MMW and HMW represents low molecular weight, middle molecular weight and high molecular weight respectively, and are industrial chemicals. BFR represents brominated flame retardants.*

Table 5-22 Comparing the degradation rates of different classes of chemicals under aerobic conditions with facultative pond inocula

Compound	Class of chemical	First order rates (d^{-1})	Class average rate (d^{-1})	First order rates (d^{-1})	Class average rate (d^{-1})
		Light		Dark	
Triclosan	PCP	0.0566	0.0566	0.1752	0.1752
Naphthalene	LMW PAHs	0.289	0.2707	0.3046	0.2645
Acenaphthylene	LMW PAHs	0.3129		0.3337	
Acenaphthene	LMW PAHs	0.3443		0.3833	
Fluorene	LMW PAHs	0.2472		0.2641	
Phenanthrene	LMW PAHs	0.2995		0.2111	
Anthracene	LMW PAHs	0.1313		0.0904	
Fluoranthene	MMW PAHs	0.0863	0.0576	0.1024	0.0620
Pyrene	MMW PAHs	0.0787		0.0947	
Benz(a)anthracene	MMW PAHs	0.0347		0.0257	
Chrysene	MMW PAHs	0.0305		0.0253	
Benzo(b)fluoranthene	HMW PAHs	0.033	0.0364	0.0173	0.0200
Benzo(a)pyrene	HMW PAHs	0.049		0.0385	
Indeno(1,2,3-cd)pyrene	HMW PAHs	0.0388		0.0169	
Dibenz(a,h)anthracene	HMW PAHs	0.0322		0.009	
Benzo(g,h,i)perylene	HMW PAHs	0.0291		0.0184	
EE2	Steroidal hormones	0.492	0.4296	0.5064	0.3900
E1	Steroidal hormones	0.4368		0.3888	
E2	Steroidal hormones	0.4872		0.5016	
E3	Steroidal hormones	0.3024		0.1632	
BDE 28	BFR	0.0666	0.0551	0.0599	0.0644
BDE 47	BFR	0.0612		0.0692	
BDE 99	BFR	0.0626		0.0648	
BDE 100	BFR	0.059		0.0677	
BDE 153	BFR	0.0575		0.0645	
BDE 154	BFR	0.0578		0.067	
BDE 183	BFR	0.0479		0.0616	
BDE 209	BFR	0.0285		0.0604	

**PCP represents personal care product; * LMW, MMW and HMW represents low molecular weight, middle molecular weight and high molecular weight respectively, and are industrial chemicals. BFR represents brominated flame retardants.*

A similar trend was observed with facultative pond inocula where the degradation of estrogens was 2 – 3, 6 – 8, 12 – 20 and 2 – 8 times faster than LMW PAHs, MMW PAHs, HMW PAHs and triclosan respectively under light and dark incubation conditions. Furthermore, the degradation of PAHs was 3 - 4 times faster than PBDEs under both incubation conditions. In summary, degradation of estrogens > PAHs > triclosan > PBDEs.

5.3.3.6 Predicting effluent quality and associated risks using obtained degradation rates

The obtained biodegradation rates were used to predict effluent concentrations and assess the likelihood of risk when these chemicals are discharged into water bodies. The method and assumptions made were previously described in Section 4.5.8. In addition to these assumptions, both the activated sludge WWTP (WWTP A) and the waste stabilization pond (WSP) were assumed to behave liked continuous stirred reactors (CSTRs). Sorption to sludge was not considered for estrogens (E1 and E3) as their low Log K_{ow} (3.13 and 2.47 respectively) indicates low potential to sorb to solids; 99 % of E1 and E3 were also observed in the aqueous phase in the biodegradation assay. HRT for WWTP A and WWTP C is 4.2 hours and 20 days respectively. Information on EQS and PNEC values for the chemicals were previously given in Section 4.5 and 5.3.2.

Table 5-23 Predicted effluent concentration of the chemicals after activated sludge aerobic treatment (Brazil WWTP A)

Compound	Measured inf conc (ng/L)	Conc after sorption (ng/L)	Rate (h^{-1})	Predicted eff conc (ng/L)	Measured eff conc (ng/L)	EQS standard (ng/L)
Triclosan	49184	7377.6	0.0072	7161.0	1486	100
Naphthalene	2109.1	316.3	0.0340	276.8	200.8	130,000
Acenaphthylene	259.3	38.8	0.0180	36.1	55.8	-
Acenaphthene	347.2	52.0	0.0165	48.7	35.6	-
Fluorene	778.0	116.6	0.0134	110.4	75.2	-
Phenanthrene	2119.1	317.8	0.0126	301.8	140.3	-
Anthracene	395.2	59.3	0.0082	57.3	67.0	100
Fluoranthene	1135.2	170.3	0.0063	165.9	95.1	120
Pyrene	1054.5	158.1	0.0038	155.6	100.5	-
Benz(a)anthracene	526.4	78.9	0.0050	77.3	90.5	-
Chrysene	459.2	68.8	0.0060	67.1	81.3	-
Benzo(b)fluoranthene	631.0	94.7	0.0053	92.7	57.7	17
Benzo(a)pyrene	393.3	59.0	0.0032	58.2	70.9	270
E1	78.2	n/a	0.1261	50.9	16.1	6^{+}
E3	1234.2	n/a	0.1426	771.6	64.3	60^{+}

-the three HMW PAHs not included were no detected in the WWTP influent
-Degradation rates were not obtained for PBDEs, hence they were excluded
- Conc represents concentration; inf and eff represents influent and final effluent respectively.
- Concentrations of the chemicals used was previously reported above
-+ are PNEC values; -n/a means not applicable

Table 5-24 Predicted effluent concentration of the chemicals after facultative pond treatment (Brazil WWTP C)

Compound	Measured inf conc (ng/L)	Conc after sorption (ng/L)	Rate (d^{-1})	Predicted eff conc (ng/L)	Measured eff conc (ng/L)	EQS standard (ng/L)
Triclosan	17797.0	2669.6	0.1752	592.7	924.0	100
Naphthalene	3674.3	551.1	0.3046	77.7	124.7	130,000
Acenaphthylene	1561.9	234.3	0.3337	30.5	47.6	-
Acenaphthene	770.8	115.6	0.3833	13.3	34.6	-
Fluorene	3987.7	598.1	0.2641	95.2	68.2	-
Phenanthrene	7658.8	1148.8	0.2111	220.0	170.6	-
Anthracene	2073.3	311.0	0.0904	110.8	71.2	100
Fluoranthene	991.4	148.7	0.1024	48.8	127.5	120
Pyrene	4073.5	611.0	0.0947	211.1	113.8	-
Benz(a)anthracene	723.3	108.5	0.0257	71.7	91.5	-
Chrysene	666.3	99.9	0.0253	66.4	82.8	-
Benzo(b)fluoranthene	541.4	81.2	0.0173	60.3	68.8	17
Benzo(a)pyrene	573.4	86.0	0.0385	48.6	66.3	270
E1	50.5	n/a	0.3888	5.8	0.1	6^{+}
E3	625.1	n/a	0.1632	146.6	13.0	60^{+}
EU WFD PBDEs	18.4	2.8	0.0655	1.2	12.8	140
BDE 209	251.2	37.7	0.0604	17.1	<10.8	4.8^{+}

-the three HMW PAHs not included were no detected in the WWTP influent
- Conc represents concentration; inf and eff represents influent and final effluent respectively.
- Concentrations of the chemicals used was previously reported in Section X
-+ are PNEC values; -n/a means not applicable

As shown in Table 5-23 and Table 5-24, the predicted effluent concentration for triclosan in both WWTP A (7161 ng/L) and WWTP C (593 ng/L) was well above the EQS value (100 ng/L). In both WWTP A and WWTP C, the predicted effluent concentration of naphthalene and benz(b)fluoranthene were below their EQS values. The predicted concentration of fluoranthene was below the EQS values in WWTP C but exceeded it in WWTP A. Also, the predicted concentration of anthracene was below the EQS in WWTP A, but exceed it in WWTP C. For estrogens, the predicted concentration of E1 was below its PNEC value in WWTP C, but exceeded it in WWTP A, while those of E3 exceeded the EQS in both WWTP A and WWTP C. The summed predicted concentration of EU Water Framework Directive (WFD) PBDEs (BDE 28, 47, 99, 100, 153, 154) was below the EQS value in WWTP C (Table 5-24). Hence, effluent from these plants would pose a risk to aquatic organisms when discharged into receiving waters.

To avert this, an impractical HRT of 10,100 h and 147 days is required to ensure the concentration of triclosan is below in the EQS value in WWTP A and WWTP C respectively. However, river dilution by 6 and 72 times might ensure compliance for effluents from WWTP C and A respectively. For benz(b)fluoranthene, an HRT of 862 hours (in WWTP A) and 218 days (in WWTP C) would be required to achieve concentrations below the EQS values; however, river dilution by 4 and 6 times will ensure compliance for effluents for WWTP A and C respectively. Fluoranthene would be removed below the EQS with a dilution factor of 1.3 times from effluent of WWTP A, while anthracene just requires a dilution of 1.1 times from WWTP C effluent. E3 requires an HRT of 137 hours or a river dilution factor of 1:13 to ensure compliance to the PNEC values in WWTP A, but requires an HRT of 58 days and river dilution of 1:2 to ensure compliance in WWTP C. E1 requires an HRT of 95 hours and a river dilution factor of 1:9 to ensure compliance for effluent from WWTP A. BDE 209 requires an HRT of 113 days or a river dilution factor of 1:4.

5.3.4 Microbial diversity and association of bacterial taxa to the biodegradation of triclosan, PAHs, estrogen and PBDEs by facultative inocula from a waste stabilization pond (WSP)

This section looks into the change in microbial community after degradation of the diverse classes of chemicals (triclosan, PAHs, estrogen and PBDEs with facultative pond inocula from WWTP C. Furthermore, the enrichment and impoverishment in the abuncance of bacterial genera in the inocula after the biodegradation assays is discussed. Also, bacterial genera that were suspected to be responsible for the biodegradation of these chemicals were identified- spiked reactors were compared to un-spiked reactors (controls) to determine this.

5.3.4.1 Inter-relationship between the micro bacterial communities

The result of the multidimensional scale plot at genus level taxonomy shows the clustering of the microbial community before and after degradation of the compounds over time (Figure 5-24). A shift in the microbial community after degradation of the compounds occurred as the libraries at the start and end of the experiment clustered differently under both illumination conditions. Furthermore, the microbial community of the control (un-spiked) reactor differed differently at the end of the experiment from the spiked reactors. This suggests that the microbial community shifted maybe due to competition of available nutrients over time and subsequent enrichment or reduction of competing genera. Additionally, the libraries under light and dark illumination conditions clustered differently and shifted in opposite directions. This indicates enrichment or reduction of different genera under both conditions.

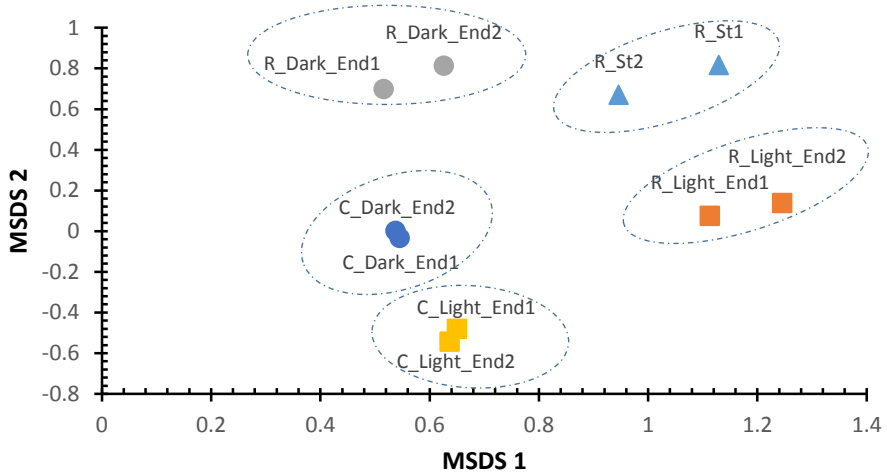


Figure 5-24 Multidimensional scale plot comparing the bacterial communities at genus level present in the aerobic batch tests before and after degradation of the selected chemicals. Clusters for duplicate samples and various stages are circled separately. C refers to the control reactor (not spiked with micropollutants) and R refer to spiked reactors. St and End represent start and end of the experiment. 1 and 2 are replicates. Light and dark represents the illumination conditions

5.3.4.2 Microbial diversity and species enrichment

Relative abundances of some bacteria genera in the facultative inocula were observed to have increased or decreased at the end of the biodegradation assay when compared to the un-spiked control. Some of the genera whose relative abundance increased significantly ($p < 0.05$, G's test) under both light and dark conditions include *Pseudoxanthomas, Gordonia, Sphingobacterium, Rhodobacter, Ralstonia, Devosia* and *Hydrogenophaga* (Figure 5-25, Figure 5-26). However, genera including *Leadbetterella, Areminomas, Nannocystics* and *Agrobacterium* were enriched only under light conditions (Figure 5-25). Meanwhile, *Rhodococcus, Achromobacter, Niabella, Burkholderia, Paludibacter* and *Methanobecterium* were enriched only in the dark (Figure 5-26).

Among these enriched genera, *Rhodococcus* has previously been identified as a versatile degrader of organic micropollutants as it has been associated with degradation of triclosan (Lee and Chu, 2013), PAHs (Peng *et al.*, 2008; Ghosal *et al.*, 2016), estrogens (Yoshimoto *et al.*, 2004; Yu *et al.*, 2013) and PBDEs (Robrock *et al.*, 2009). Also, *Burkholderia* has been reported to metabolise low-middle molecular weight PAHs (Peng *et al.*, 2008), and tri-hexa brominated PBDEs (Robrock *et al.*, 2009). *Gordonia* (Gallego *et al.*, 2014) and *Agrobacterium (Ghosal et al., 2016)* have also been associated with degradation of PAHs while *Ralstonia* has been reported to degrade PAHs (Seo *et al.*, 2009) and estrogens (Weber *et al.*, 2005). Furthermore, *Hydrogenophaga, Niabella* and the phototropic bacterium - *Rhodobacter* have also been reported with the ability to degrade PAHs (Oberoi *et al.*, 2015; Jiao *et al.*, 2016). Meanwhile, *Sphingobacterium* has been associated with the metabolism of estrogens- E1, E2, E3 and EE2 (Haiyan *et al.*, 2007). The difference in the enriched microbial community under light and dark conditions might explain why certain compounds degraded better under each condition. For example, the high enrichment of *Rhodococcus* under dark conditions only might explain the observed better triclosan removal rate. On the other hand, estrogens might have degraded better under light conditions due to the enrichment of *Sphingobacterium.*

There was also a significant difference ($p < 0.05$) in the reduction of the relative abundance of some genera after the biodegradation assay (Figure 5-25, Figure 5-26). Interestingly, among those with reduced abundance was *Pseudomonas* - a genus widely known to possess catabolic capability to degrade a wide range of xenobiotic compounds (Peng *et al.*, 2008).

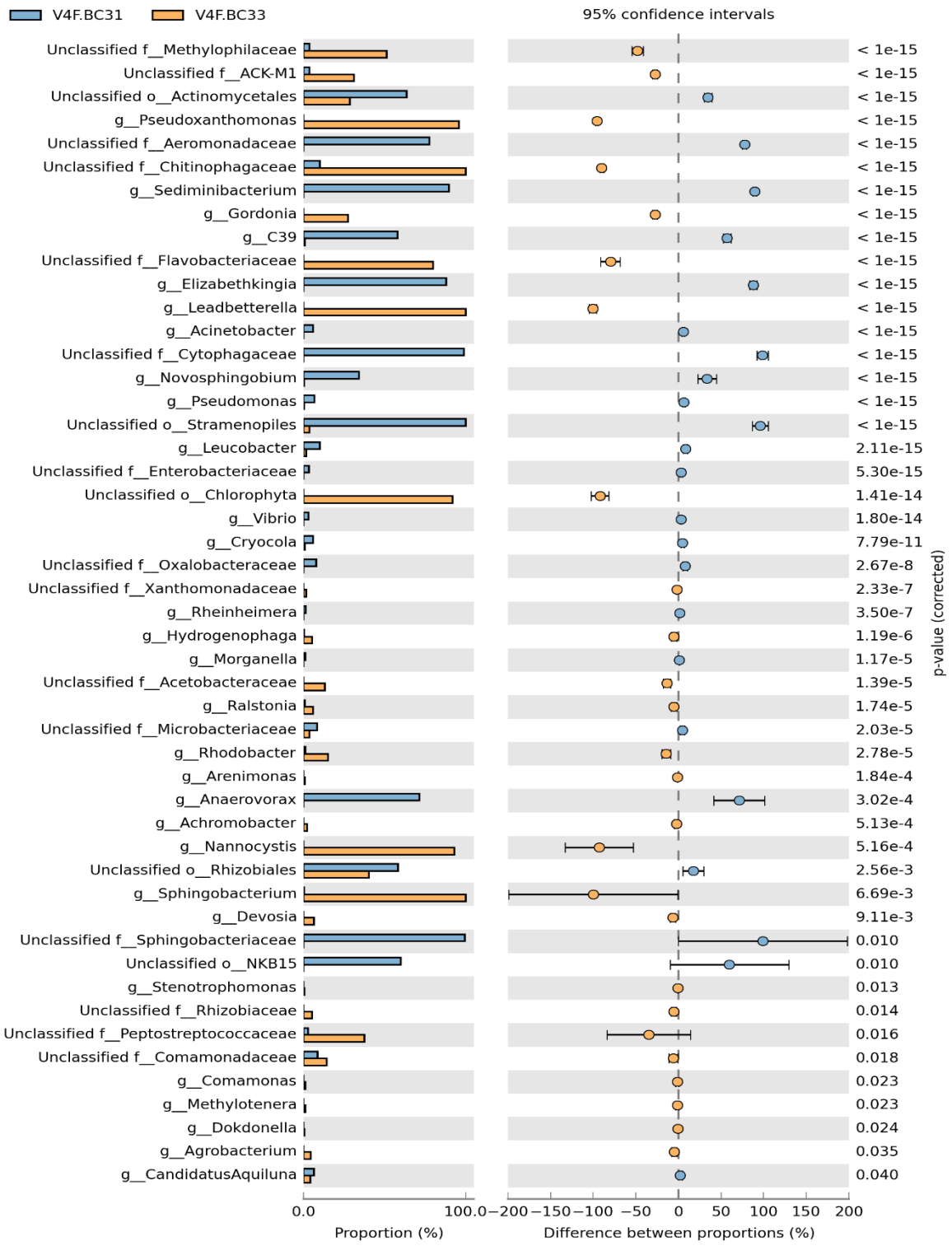


Figure 5-25 Extended error bar plot comparing the relative abundance of metagenomics profile for micropollutants degradation test using facultative inocula (Genus profile level to Class parent level). Significantly different genera (P-value < 0.05, G-test + Fisher) between the start and end of the experiment under light conditions were indicated. BC31 and BC33 represents start and end of the experiment respectively

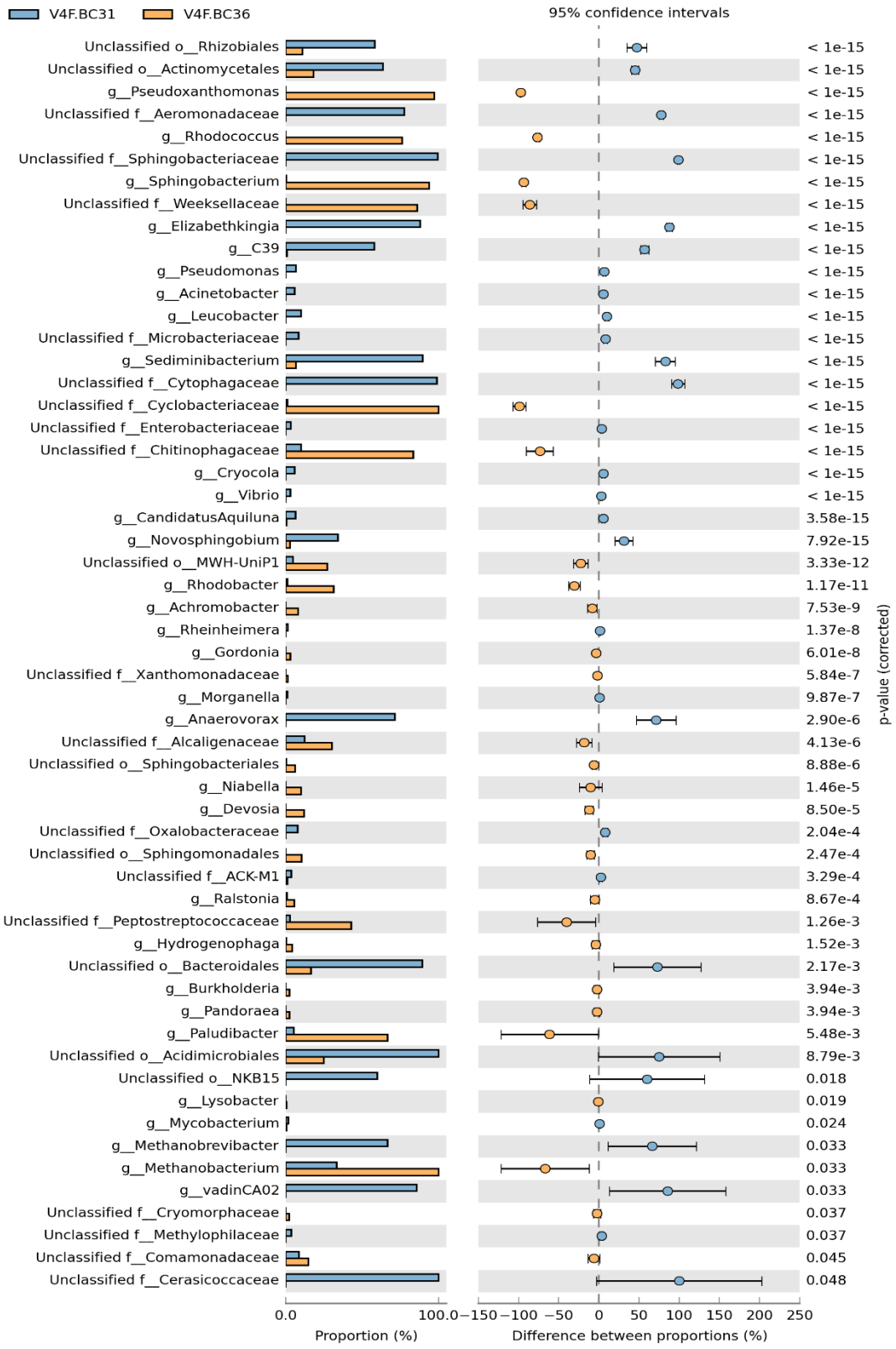


Figure 5-26 Extended error bar plot comparing the relative abundance of metagenomics profile for micropollutants degradation test using facultative inocula (Genus profile level to Class parent level). Significantly different generas (P-value < 0.05, G-test + Fisher') between the start and end of the experiment under dark conditions were indicated. BC31 and BC36 represents start and end of the experiment respectively

Additionally, a ranking of the OTUs at genus taxonomy level based on their relative abundance before and after degradation of the chemicals showed an increase in the relative abundance and rank of the significantly enriched genera and vice versa (Figure 5-27). Some known degraders of these chemicals- *Rhodococcus*, *Gordonia*, *Agrobacterium* and *Rhodobacter* were significantly enriched and resultantly, their rank order improved. On the other hand, some other known degraders- *Pseudomonas* and *Novosphingobium* reduced in abundance and their rank order diminished (Figure 5-27). The rank order of these significantly changed genera were also different under light and dark incubation conditions. For example, the rank order of *Rhodococcus* changed from 129 to 51 and 2 under light and dark conditions respectively.

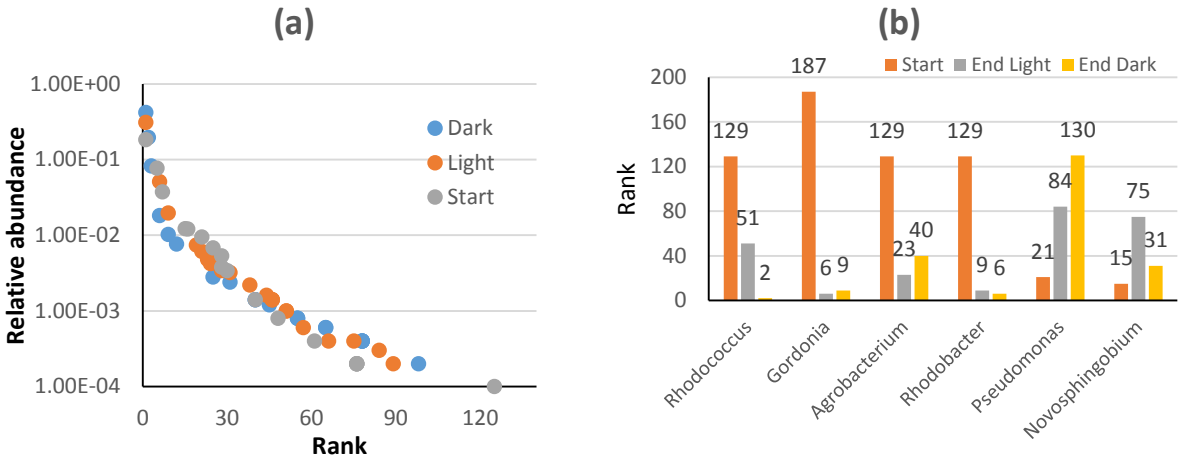


Figure 5-27 Relative abundance of significantly changed genera after the degradation of the selected chemicals in rank order. (b) The rank order of some known degraders of this chemicals at the start and end of the degradation assay under light and dark conditions

5.4 Conclusion

This study has investigated the fate of twenty-eight chemicals from four classes (biocide-triclosan, industrial chemical- 15 PAHs, steroidal hormones- 4 estrogens, and flame retardants-8 PBDEs) for the first time in Brazil. The concentrations of triclosan, PAHs, estrogens and PBDEs were similar to those in HIC and LMICs and there appears to be no pattern amongst such countries. This suggests that such chemicals are in widespread use.

This study also aimed to investigate the fate of such pollutants in three types of WWTP that differ in their energy requirements and types of processes including; energy-intensive aerobic systems (activated sludge), low-energy anaerobic-aerobic systems (UASBs and trickling filters) and passive-energy facultative systems (waste stabilization ponds). In addition the potential mechanisms and rates were studied using degradation experiments with appropriate controls. In most cases, and for all the chemical classes, the least energy intensive and cheapest WSP system (WSP) outperformed (89 – 99 % removal) or matched the high energy activated sludge system (WWTP A) (79 – 94 % removal), therefore showing its potential for micropollutant removal. However, despite the removal achieved by these systems, the concentration of triclosan and estrogens were above their PNEC value, therefore might pose risk to aquatic organisms when discharged.

Also, the compounds degraded faster under aerobic conditions with activated sludge, with first order degradation kinetics in the following decreasing order estrogens > LMW PAHs > triclosan > MMW PAHs > HMW PAHs (experiment for PBDEs was not carried out). Degradation was also observed with the facultative pond inocula under aerobic conditions, and the degradation kinetics generally was in the following decreasing order estrogens > LMW PAHs > triclosan > PBDEs > MMW PAHs > HMW PAHs. The result suggested biodegradation as a major removal mechanism during biological treatment in WWTP A and WWTP C. However, sorption of the compounds to biosolids during the experiment suggests that adsorption also plays an important role in their removal in this WWTP, while volatilization might have contributed to the removal of low molecular weight PAHs. Furthermore, methyl triclosan formed concomitantly with triclosan degradation, which also explains its detection in the WWTPs. Interestingly, the compounds behave differently in the photodegradation study as triclosan and estrogens degraded faster under light conditions (white fluorescent light) while PAHs and PBDEs degraded faster in the dark. This was due to their different physio-chemical properties and the competition within the microbial community in the inocula under the light and dark conditions. Additionally, degradation of all

the compounds was not observed under anaerobic conditions with UASB sludge inocula suggesting that their removal – if any – during UASB treatment in WWTP B was most likely attributed to sorption onto sludge and not biodegradation. Taxonomic analysis showed a significant change in the metagenomic profile in the inocula and the bacterial libraries clustered differently under light and dark conditions after the biodegradation assay with facultative inocula under aerobic conditions. Several genera known to degraders of these chemicals including *Rhodococcus*, *Burkholderia*, *Gordonia*, *Ralstonia*, *Hydrogenophaga* were enriched significantly after the biodegradation assay and their rank abundance increased when compared to the un-spiked control.

The predicted effluent concentrations from the obtained first order degradation rates showed that triclosan concentration in WWTP A and WWTP C was above the EQS value and HRT required for removal is impractical. Hence, this effluent would pose risk to aquatic organisms when discharged into receiving waters- unless the river dilutes effluent from WWTP A and WWTP C by 72 and 6 times respectively. Benzo(b)fluoranthene will require a river dilution factor of 1:4 and 1:6 from effluents from WWTP A and WWTP C respectively to ensure compliance to the EQS values. Estrogens will require a dilution factor of 1:2 to 1:3 to ensure compliance from effluent of both WWTPs, while PBDEs will require a dilution factor of 1:4 to 1:9. Although, the synergistic effect of the cocktail of different classes of compounds on aquatic organisms remains unknown.

Chapter 6: UK vs Brazil- Concentrations and Rates

Chapter 6 Comparing the concentration and extent of degradation of micropollutants between the UK and the Brazilian activated sludge WWTP

6.1 Introduction

In activated sludge systems, microbial catabolic potential, plant configurations and operating conditions influence the removal of micropollutants. Parameters such as sludge retention time, hydraulic retention time, redox conditions, pH and temperature influence micropollutant removal efficiencies in biological wastewater treatment systems (Luo *et al.*, 2014). Hence, this chapter compares the removal of triclosan and PAHs between a UK and Brazilian activated sludge plant that may contain different microbial populations, operate under different dissolved oxygen concentrations and are fed with wastewater with different temperatures. The UK plant operated under fully aerobic conditions while the Brazilian plants operated at low oxygen conditions. Furthermore, the average temperature of the wastewater in UK and Brazil is 12 – 15 ^{o}C and 25 ^{o}C respectively. Anaerobic systems were not included in this study as there was little or no degradation of the investigated chemicals in these systems.

This chapter also provides insight into the potential effect of temperature on the degradation of triclosan, PAHs and estrogens experiments carried out in Section Chapter 4 and Chapter 5 in the UK and Brazil respectively. The degradation experiment was carried at a controlled temperature of 20 ^{o}C in the UK, while in Brazil the experiment was performed at room temperature in the summer season (average temperature was 27 ^{o}C). Temperature greatly affects biological reactions in wastewater treatment (Verlicchi *et al.*, 2012), and the rate of biological reactions generally increases with rising temperature until maximum rate and thermal independence is achieved (Bennett, 1984). According to the Arrhenius equation, the rate constant of a chemical reaction is strongly dependent on temperature; such that the reaction rate increases exponentially with temperature (Bennett, 1984). This generalised empirical relationship has largely represented chemical and enzyme reaction rates, but has also been appropriated by microbiology to compare rates of reactions in populations (Koutsoumanis *et al.*, 2000; Huang *et al.*, 2011). Hence, this study aimed to compare reaction rates to explore if they are in line with empirical relationships between rates and temperature according to the Arrhenius equation. Understanding the effect of temperature on the rate of micropollutant degradation will further our knowledge on parameters essential for effective micropollutant removal.

6.2 Methodology

6.2.1 Plant characteristics

The activated sludge grab sample used for the UK triclosan and PAH degradation experiment had a total suspended solids (TSS) and volatile suspended solids (VSS) content of 3.3 g/L and 2.3 g/L respectively, while that used for estrogen degradation had a TSS and VSS content of 1.92 g/L and 1.54 g/L respectively. Both sludge samples were collected from different nitrifying treatment plants in North East England with operating at dissolved oxygen concentration of 2 – 3 mg/L. The activated sludge samples used for the Brazil degradation experiments had a TSS and VSS content of 4.2 g/L and 3.8 g/L respectively. This treatment plant in South East, Brazil was not a nitrifying plant and operated with a dissolved oxygen concentration of 0.2 – 0.3 mg/L.

6.2.2 Quantification of compounds in wastewater

The sampling, extraction and analytical method for the compounds have been presented earlier in Chapter 3. The concentrations of these compounds in the total phase (particulate matter and aqueous phase) and aqueous phase have also been presented in Chapter 3 and Chapter 5.

6.2.3 Rate comparison

The average incubation temperature for the UK and Brazil degradation experiment was 20 ^{o}C and 27 ^{o}C respectively. To compare the reaction kinetics for the degradation of the compounds under these different temperatures, the rates for the UK experiment was estimated at 27 ^{o}C based on the assumption that the temperature influences the metabolic activities of microbial population, and hence the reaction kinetics (Tchobanoglous *et al.*, 2014).

$$\left(\frac{K_2}{K_1}\right) = \theta^{(T2-T1)}$$

Where K_1 and K_2 = rates at 15 ^{o}C and 20 ^{o}C respectively, T1 and T2 are temperatures 15 ^{o}C and 20^{o}C respectively (in Celsius or Kelvin), and θ is the temperature activity coefficient.

The temperature activity coefficient for most biological systems ranges from 1.02 to 1.10 (Tchobanoglous *et al.*, 2014). Hence, a θ value of 1.08 was assumed to calculate the degradation rates for estrogens at 20 ^{o}C

6.3 Results and discussion

6.3.1 Concentrations of triclosan and PAHs in UK and Brazilian WWTP

The total (particulate and aqueous) concentration of triclosan in the influent of the Brazilian WWTP (49 µg/L) was about four times higher than those found in the UK WWTP (13 µg/L) (Table 6-1). However, similar concentrations were found in the aqueous phase of both plants (1.3 – 1.4 µg/L) (Table 6-2). The total concentration of PAHs in the influent of the Brazilian WWTP (10.2 µg/L) was also higher than that in the UK WWTP (6.6 µg/L). However, low molecular weight PAHs were the most abundant in the Brazilian influent, while middle molecular weight PAHs were the most abundant in the UK (Table 6-1). Dibenz(a,h)anthracene and benzo(ghi)perylene were only detected in the UK WWTP but were below the method quantification limit in the Brazilian WWTP (Table 6-1, Table 6-2).

The total removal rate for triclosan was higher in the Brazil WWTP (97 %) compared to the UK WWTP (89 %) (Table 6-1). However, the aqueous phase removal was higher in the UK WWTP (89 %) (Table 6-2Table 6-2). Similarly for PAHs, the Brazilian WWTP removed more individual and total PAHs from the total phase (90 % compared to 72 %), but removed less in the aqueous phase when compared to the UK WWTP (13 % compared to 76 %). The total phase concentration of triclosan in the treated effluent of both WWTP was similar (1.4 – 1.5 µg/L) (Table 6-1), however, the aqueous phase concentration was five times higher in the Brazilian WWTP (Table 6-2). For PAHs, the total phase concentration was higher in the UK WWTP (1.8 µg/L compared to 1.0 µg/L in Brazil) (Table 6-1), but the aqueous phase concentration was two times higher in the Brazilian WWTP (Table 6-2).

Table 6-1 Total (particulate and aqueous phase) concentrations of triclosan and 15 PAHs in the influent and effluent from a UK and Brazilian activated sludge WWTP.

Compound	UK WWTP			Brazilian WWTP		
	Influent (ng/L)	Effluent (ng/L)	% Removal	Influent (ng/L)	Effluent (ng/L)	% Removal
Triclosan	13117 (1030)*	1443 (43)	88.9	49184 (7429)	1486 (9)	97.0
PAHs						
Naph	689.4 (44.5)	211.1 (25.2)	69.5	2108.9 (86.0)	200.8 (13.4)	90.5
Acy	191.5 (15.7)	63.3 (3.0)	66.9	258.8 (11.3)	55.8 (0.5)	78.4
Ace	182.9 (24.9)	35.1 (5.0)	81.0	346.9 (11.4)	35.6 (1.4)	89.7
Flu	277.8 (33.0)	47.8 (1.4)	82.8	777.5 (15.3)	75.2 (9.1)	90.3
Phen	633.7 (61.1)	87.7 (21.3)	86.2	2118.8 (64.0)	140.3 (9.9)	93.4
Anth	421.1 (18.2)	95.3 (8.3)	77.3	395.3 (5.8)	67.0 (1.6)	83.1
Flt	743.1 (46.5)	101.4 (2.0)	86.4	1135.0 (21.6)	95.1 (7.6)	91.6
Pyr	691.2 (21.9)	99.8 (1.0)	85.6	1053.7 (1.8)	100.5 (6.1)	90.5
BaA	754.4 (47.6)	181.3 (0.4)	76.0	526.3 (9.7)	90.5 (0.4)	82.8
Chry	556.2 (58.5)	129.5 (0.6)	76.7	458.6 (5.0)	81.3 (0.5)	82.3
BbF	238.6 (58.5)	65.4 (11.1)	72.6	631.5 (18.5)	57.7 (1.1)	90.9
BaP	355.3 (75.7)	266.3 (31.2)	22.9	393.0 (10.7)	70.9 (3.4)	82.0
InPy	-	-	-	-	-	-
DiahA	590.1 (179)	273.5 (24.5)	53.7	-	-	-
BghiP	241.8 (44.1)	210.2 (35.4)	13.2	-	-	-
∑LMW PAHs	2396.3	539.7	77.5	6006.2	574.7	90.4
∑MMW PAHs	2744.3	511.3	81.4	3173.6	367.4	88.4
∑HMW PAHs	1425.9	822.7	42.3	1024.5	128.7	87.4
∑PAHs	6566.5	1873.7	71.5	10204.3	1070.7	89.5

*-PAHs abbreviation- naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (pyr), benz(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InPy), dibenz(a,h)anthracene (DiahA), and benzo(ghi)perylene (BghiP); *Standard deviation in bracket; n = 3*

Table 6-2 Aqueous phase concentrations of triclosan and 15 PAHs in the influent and effluent from a UK and Brazilian activated sludge WWTP

Compound	UK WWTP			Brazilian WWTP		
	Influent (ng/L)	Effluent (ng/L)	% Removal	Influent (ng/L)	Effluent (ng/L)	% Removal
Triclosan	1391 (58)	142 (6.2)	89.0	1303 (223)	549 (24)	58
PAHs						
Naph	108.6 (20.4)	16.3 (0.5)	85.0	114.3 (6.0)	46.1 (4.0)	59.7
Acy	39.6 (2.5)	12.3 (0.2)	68.9	20.2 (0.7)	23.3 (0.9)	0.0
Ace	45.0 (5.0)	4.7 (0.1)	89.6	19.6 (0.4)	13.5 (0.6)	31.1
Flu	48.0 (3.4)	8.4 (0.3)	82.4	44.2 (0.8)	29.6 (2.2)	33.0
Phen	77.7 (0.7)	10.2 (0.1)	86.9	65.7 (4.5)	43.1 (5.3)	34.4
Anth	47.4 (1.4)	19.2 (1.6)	59.4	26.6 (0.6)	28.2 (1.2)	0.0
Flt	75.0 (2.8)	18.6 (0.2)	75.2	42.4 (1.0)	35.8 (2.8)	15.8
Pyr	68.3 (1.5)	18.2 (0.1)	73.3	39.9 (1.4)	38.0 (2.4)	4.6
BaA	111.1 (1.2)	36.2 (0.1)	67.4	37.5 (0.3)	38.2 (1.7)	0.0
Chry	68.2 (1.8)	25.5 (0.7)	62.6	32.8 (0.3)	34.0 (0.9)	0.0
BbF	15.7 (3.1)	6.3 (1.1)	59.8	24.4 (0.4)	26.8 (2.5)	0.0
BaP	12.9 (3.4)	4.2 (1.1)	67.5	25.8 (0.3)	27.6 (3.6)	0.0
InPy	91.4 (4.9)	11.6 (4.6)	87.3	-	-	-
DiahA	39.1 (9.1)	7.7 (2.1)	80.3	-	-	-
BghiP	11.3 (0.4)	10.0 (3.0)	11.5	-	-	-
∑LMW PAHs	366.2	71.1	80.6	290.7	183.9	36.7
∑MMW PAHs	322.5	98.6	69.4	152.6	146.0	4.3
∑HMW PAHs	170.4	39.8	76.7	50.3	54.4	-8.2
∑PAHs	859.1	209.4	75.6	553.5	483.7	12.6

*PAHs abbreviation- naphthalene (Naph), acenaphthylene (Acy), Acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (pyr), benz(a)anthracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InPy), dibenz(a,h)anthracene (DiahA), and benzo(ghi)perylene (BghiP).; *Standard deviation in bracket; n = 3*

6.3.2 **Comparing degradation rates in the UK and Brazil experiments**

The Arrhenius relationship was not followed in this study as the disappearance rates of most of the chemicals were lower at a higher temperature (Table 6-3, Table 6-4). Important variables that might have influenced the reaction rate include; microbial taxa/catabolic potential present in the inocula, the dissolved oxygen (DO) concentration and the pH (Verlicchi *et al.*, 2012). These parameters differed considerably between the UK and Brazil experiment except the pH of both systems (pH 5 – 7). It seems unlikely that the same putative degraders were involved in the degradation of the chemicals in both locations. The DO concentration was 4 – 7 mg/L and 0.3 – 0.5 mg/L respectively for the UK and Brazil. The DO concentrations in the UK and Brazil experiment were maintained at the same concentrations used in the WWTPs to replicate operating conditions. It is important to note that the disappearance rate of some LMW PAHs was due to biodegradation and volatilization as earlier discussed in Section 4.5.4.1, and 5.3.3.2.1. Volatilization was observed to contribute more to the removal of LMW PAHs in the Brazil experiment as the estimated volatilization rates were much higher (Table 6-5). This might also be due to the low DO concentration and higher temperature in the Brazil experiment that favoured the volatilization process.

Table 6-3 Comparing first order degradation rates for triclosan and PAHs under aerobic conditions with activated sludge inocula from UK and Brazil

Chemical	UK rates 20°C (h^{-1})	UK rates 27°C (h^{-1})	Brazil rates (h^{-1})
Triclosan	0.0071	0.0120	0.0072
Naphthalene	0.0631	0.1080	0.0340
Acenaphthylene	0.0238	0.0410	0.0180
Acenaphthene	0.0291	0.0500	0.0165
Fluorene	0.0225	0.0390	0.0134
Phenanthrene	0.0210	0.0290	0.0126
Anthracene	0.0172	0.0220	0.0082
Fluoranthene	0.0130	0.0220	0.0063
Pyrene	0.0115	0.0200	0.0038
Benz(a)anthracene	0.0067	0.0110	0.0051
Chrysene	0.0060	0.0100	0.0061
Benzo(b)fluoranthene	-	-	0.0055
Benzo(a)pyrene	-	-	0.0034
Indeno(1,2,3-cd)pyrene	-	-	0.0032
Dibenz(a,h)anthracene	-	-	0.0022
Benzo(ghi)perylene	-	-	0.0024

Average incubation temperature for the UK and Brazil experiment was 20 and 27 °C respectively. The last 5 PAHs did not degrade in the UK experiment, so do not have comparative rates

Table 6-4 Comparing first order degradation rates for estrogens under aerobic conditions with activated sludge inocula from UK and Brazil

Chemical	UK rates 20 ºC (h^{-1})	UK rates 27 ºC(h^{-1})	Brazil rates (h^{-1})
EE2	0.1415	0.2425	0.0331
E1	0.1760	0.3017	0.1261
E2	0.2990	0.5124	0.1496
E3	1.3137	2.2515	0.1426

Average incubation temperature for the UK and Brazil experiment was 20 and 27 ^{o}C respectively

Table 6-5 Comparing volatilization rate of LMW PAHs in the UK and Brazil aerobic degradation experiment at 27^{o}C

	UK experiment			Brazil Experiment		
Chemical	**Total (h^{-1})**	**Vol (h^{-1})**	**% vol**	**Total (h^{-1})**	**Vol (h^{-1})**	**% vol**
Naphthalene	0.1081	0.0360	33.3	0.034	0.0291	85.6
Acenaphthylene	0.0408	0.0050	12.2	0.018	0.0167	92.8
Acenaphthene	0.0499	0.0060	12.0	0.0165	0.0147	89.1
Fluorene	-	-	-	0.0134	0.0063	47.0

**vol represents volatilization*

The reaction rates in the Brazil experiment were 2 – 20 times slower than the UK experiment despite the higher temperature. As well as the different biology, the lower rates from the Brazil inocula may have been compounded by the low dissolved oxygen concentration of the Brazil sludge (0.3 mg/L) compared to the UK sludge (4 mg/L) as degradation of micropollutants has been reported to be faster under nitrifying conditions (aerobic conditions) than non-nitrifying conditions (Verlicchi *et al.*, 2012). In fact, higher micropollutant removal efficiencies have been suggested to occur in plants with high nitrogen removal capabilities (Batt *et al.*, 2006; Vieno *et al.*, 2007). Indeed, the presence of nitrifying and ammonia oxidizing bacteria that have been found to possess high cometabolic capability on a wide range of persistent compounds (Batt *et al.*, 2006; Haiyan *et al.*, 2007). This also explains the better removal rate of triclosan and PAHs in the aqueous phase (aqueous phase removal mostly by biodegradation as compounds are more bio-available) by the nitrifying UK activated sludge WWTP compared to the non-nitrifying Brazilian plant (Table 2). 58 % and 13 % triclosan and PAHs removal respectively was achieved in the Brazilian WWTP compared to 89 % and 76 % in the UK WWTP. The theoretical oxygen demand required to completely oxidize triclosan, PAHs and estrogens calculated according to OECD method 301 (Oecd, 1994); was approximately 1.3 mg/1 mg of triclosan, 0.6 mg/0.2 mg of PAHs and 0.3 mg/0.1 mg of estrogens respectively- according to the concentrations of the chemicals spiked

(see calculation in Appendix E- 8.6). This shows that the DO was limiting for triclosan and PAHs in the Brazilian WWTP (0.3 mg/L DO), but was just enough for estrogens.

6.4 Conclusion

The levels of triclosan and PAHs was higher in Brazil than in the UK, and the Brazilian activated sludge WWTP achieved a higher removal rate for both group of compounds in the total phase (particulate and aqueous phase). The UK and Brazil rates did not conform to empirical Arrhenius relationships due to the difference in putative degraders involved in degradation of the chemicals and dissolved oxygen concentrations in both locations. The superior removal of these compounds in the aqueous phase in the UK WWTP over the Brazilian WWTP is as a result of the higher UK rates.

Chapter 7: General Conclusion

Chapter 7 General conclusions

7.1 Method development and validation

Analytical methods using solid phase extraction (SPE) for sample concentration and analyte extraction, and gas chromatography (GC) coupled with mass spectrophotometry (MS) or electron capture detector (ECD) for detection and quantification were developed and validated. A novel SPE-GC-ECD method was developed and validated to quantify eight PBDE congeners in wastewater in one run. The method detection limit (0.2 – 10.8 ng/L) was low and could quantify PBDEs below proposed environmental quality standard (EQS) values and is 2 – 4 times cheaper than current GC-MS methods- therefore opens up PBDEs analysis to low-middle income countries (LMICs) where environmental analysis is limited due to associated prohibitive costs. Also, SPE-GC-MS methods were developed and validated to quantify triclosan and 15 PAHs in both aqueous and particulate matter phase of wastewater. The method detection limits (5.6 ng/L for triclosan, 0.2 – 10 ng/L for PAHs) were low and could be used to quantify triclosan and PAHs below proposed EQS values. These SPE-GC-ECD and SPE-GC-MS methods were employed in the quantification of these chemicals in UK and Brazilian WWTPs.

7.2 Occurrence and fate of micropollutants in low and high energy WWTPs in the UK and Brazil

Triclosan, PAHs, estrogens and PBDEs were detected at similar concentrations in UK and Brazilian wastewater, and were similar to those reported in other countries. Hence, these chemicals are equally used in both high income countries (HICs) and LMICs and their intrusion into the environment is a global burden. Ineffective triclosan, PAHs, and estrogens removal was observed in all WWTPs in both countries as the concentration of triclosan, some PAHs (e.g. fluoranthene, benzo(b)fluoranthene), estrogens (E1 and E3), and PBDEs (BDE 209) were above the proposed EQS values set by the EU Water Framework Directive and existing Predicted no-effect concentrations (PNECs- for estrogens and BDE 209). This implies that effluents from these WWTPs poses risk to aquatic organisms when discharged into receiving waters. Aside the risks posed by triclosan, PAHs, estrogens and PBDEs individually, the mixture effects of this cocktail of chemicals on aquatic organisms is unknown.

Furthermore, among the technologically different WWTPs, the passive-energy and cheapest waste stabilization pond (WSP) system interestingly showed high micropollutant removal by outperforming the high energy aerobic activated sludge system, while the anaerobic UASB with trickling filter system was the least effective. This shows the potential of WSPs for micropollutant removal especially in LMICs countries where sunlight, temperature and land availability is often not limiting. Furthermore, the difference in chemical structure and functional groups among the different classes of compounds was not observed to affect their removal in the three WWTPs as all the compounds were removed to a similar extent (about 90 % for each class of chemical).

7.3 Removal mechanisms of micropollutants in UK and Brazil WWTPs, and risk assessment

Under aerobic conditions, biodegradation of the chemicals was observed and the experimental first-order rates obtained for the different classes of chemicals with activated sludge inocula showed that the degradation kinetics were in the following decreasing order; estrogens >LMW PAHs > triclosan > MMW PAHs > HMW PAHs. A similar result was obtained using inocula from a facultative pond as degradation kinetics were in the following decreasing order; estrogens > LMW PAHs > triclosan > PBDEs > MMW PAHs > HMW PAHs- except for the inclusion of PBDEs that were not investigated in the activated sludge experiment. This observed degradation of these chemicals in the batch tests shows that biodegradation contributed majorly to the removal of these chemicals in both activated sludge and WSP systems. The physio-chemical properties (functional groups, number of aromatic rings, etc.) of the different classes of chemicals was observed to influence their degradation kinetics. Furthermore, this difference in physio-chemical properties also influenced their reaction kinetics in the photodegradation study; triclosan and estrogens degraded faster in the presence of white light, while reverse was the case for PAHs and PBDEs. Additionally, the importance of bacterial taxa present in the inocula and dissolved oxygen (DO) concentration and over temperature was established in degradation study, as the chemicals degraded much faster with the UK aerobic sludge compared to the Brazil low DO sludge despite a 7^{o}C lower incubation temperature. Sorption would contribute majorly to the removal of these classes of chemicals (less for E1 and E3) due to their observed high association with biosolids in our study. Volatilization was also observed to contribute to the removal of low molecular weight PAHs in the activated sludge experiments.

Additionally, all compounds were resistant to anaerobic degradation in the UK and Brazil experiments. Hence, there was no evidence of reductive dehalogenation for PBDEs and triclosan. This implies that removal of these compounds in WWTPs that use anaerobic processes (such as UASBs) will mostly be due to sorption to solids. Lastly, the predicted effluent concentrations of triclosan, some PAHs, BDE 209, and estrogens (E1 and E2) from the experimentally determined degradation rates (for the aerobic systems) in this study were above the EQS/PNEC values. It will be impossible for all the investigated treatment plants to comply with these standards, as the required hydraulic retention times are impractical.

7.4 Effect of micropollutant degradation on the bacterial taxa in the inocula

Molecular analysis showed that after the aerobic degradation assay using activated sludge spiked with PAHs, there was a significant change in the metagenomics profile of the inocula; some known PAH degrading genera such as *Mycobacterium* and *Acidiphilium* were significantly enriched compared to the non-inoculated controls. Also, after the aerobic degradation assay using facultative inocula spiked with triclosan, PAHs, estrogens and PBDEs, a significant change in the metagenomic profile of the inocula was observed, and several genera known to degrade these group of compounds including *Rhodococcus, Burrkholderia*, *Gordonia*, *Ralstonia*, *Hydrogenphaga* were significantly enriched. Consequently, enriching microbial inocula with the known degrading taxa might improve degradation of these chemicals in the studied biological systems.

7.5 Broader implications of current research and outlook

This research has contributed to knowledge by reporting the limits of micropollutant removal in biological wastewater treatment systems utilizing different technologies (including activated sludge, UASB with trickling filters and WSP). The experimentally determined rates (for the activated sludge and WSP systems) can be used to imply the kind of hydraulic retention times (HRTs) and/or river dilutions that would be required to ensure compliance to the recommended safety standards.

None of the WWTPs removed triclosan, estrogens, and some PAHs below proposed safe levels; the low energy WSP system showed a high micropollutant removal potential by achieving a better removal rate of triclosan, estrogens, PAHs and PBDEs than the energy intensive activated sludge systems. Consequently, these low energy systems can be used solely to remove micropollutants in countries with a warm climate if optimized, or can be used in-line with an activated sludge system (where land available is not limiting) to achieve

effluents below proposed discharge standards. Furthermore, treatment systems that operate under anaerobic conditions have the least potential to biologically remove micropollutants as the studied compounds were resistant to anaerobic biodegradation. Hence, focus should be on aerobic systems to remove micropollutants via biological means.

Hence my approach and proposal for sustainable micropollutant removal involves; (a) Predicting effluent concentrations using experimentally determined rates to know the true limits and feasibility of removal of specific chemicals in biological treatment systems. (b) Since most of the investigated chemicals are hydrophobic and are highly associated with suspended solids, less expensive tertiary treatment systems such as sand filters can be used to further remove these chemicals from secondary effluents. WSPs that have been shown to possess micropollutant removal potential can also be used in place of sand filters where land availability is not limiting- especially in LMICs.

7.6 Future work and recommendations

Due to the costs associated with wastewater treatment, the increased pressure of removing potential hazardous chemicals, and the difficulty of doing so without increasing energy use- as currently advocated tertiary treatment technologies (e.g. advanced oxidation processes) are high energy and carbon intensive; other cheaper technologies such as sand filters, biochar, and Fenton's reactions should be investigated for sustainable micropollutant removal.

The sampling method used is important in assessing the fate of micropollutants in WWTPs. In this study, the grab sampling method employed has the limitation of not truly representing plant performance as it does not take into account the hydraulic retention time of the plant. Therefore, a 24 or 12 hours continuous sampling across the different stages of the treatment system will provide a more accurate indication of the fate of these compounds, and better assess plant performance.

Furthermore, the biodegradation pathways for the compounds and biotransformation products under aerobic conditions should be studied in both lab scale experiments and full scale WWTPs. This is essential to ensure complete mineralization of the compound or monitor the toxicity and persistence of the formed metabolites. Furthermore, in this study bacterial generas that were enriched after degradation of the test compounds were identified. However, more molecular analysis should be carried out to identify the key genes responsible for degradation of these compounds. This knowledge would be useful to optimize the microbial community in the bioreactors- thereby improving biodegradation of micropollutants.

Also, in waste stabilization ponds, micropollutants are also removed via photodegradation. This study investigated this mechanism slightly by looking at the effect of white light on the degradation of the compounds. However, there are different solar irradiation spectrum including UV-A, UV-B, UV-C and visible light. Hence, studying the degradation of these compounds under different irradiation spectra or sunlight directly will provide more comprehensive information on the effect of photodegradation.

Lastly, based on literature and data from our study, these compounds sorb onto sludge during primary and secondary treatment, and their fate during anaerobic degradation of sludge is largely unknown. Therefore, it is important to identify the potential risks triclosan, estrogens, PAHs and PBDEs pose when this sludge is applied as manure in farmlands.

References

Ahn, M.-Y., Filley, T.R., Jafvert, C.T., Nies, L., Hua, I. and Bezares-Cruz, J. (2006) 'Photodegradation of decabromodiphenyl ether adsorbed onto clay minerals, metal oxides, and sediment', *Environmental Science & Technology*, 40(1), pp. 215-220.

Alda, M.J. and Barceló, D. (2001) 'Review of analytical methods for the determination of estrogens and progestogens in waste waters', *Fresenius' journal of analytical chemistry*, 371(4), pp. 437-447.

Alonso-Gutiérrez, J., Figueras, A., Albaigés, J., Jiménez, N., Vinas, M., Solanas, A.M. and Novoa, B. (2009) 'Bacterial communities from shoreline environments (Costa da Morte, Northwestern Spain) affected by the Prestige oil spill', *Applied and Environmental Microbiology*, 75(11), pp. 3407-3418.

Alvarino, T., Suarez, S., Lema, J.M. and Omil, F. (2014) 'Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors', *Journal of hazardous materials*, 278, pp. 506-513.

Andersen, H.R., Kjølholt, J., Hansen, M., Stuer-Lauridsen, F., Blicher, T.D., Ingerslev, F. and Halling-Sørensen, B. (2004) 'Degradation of estrogens in sewage treatment processes', *Environmental Project*, 899.

Aranami, K. and Readman, J.W. (2007) 'Photolytic degradation of triclosan in freshwater and seawater', *Chemosphere*, 66(6), pp. 1052-1056.

Armstrong, D.L., Lozano, N., Rice, C.P., Ramirez, M. and Torrents, A. (2018) 'Degradation of triclosan and triclocarban and formation of transformation products in activated sludge using benchtop bioreactors', *Environmental research*, 161, pp. 17-25.

Balmer, M.E., Poiger, T., Droz, C., Romanin, K., Bergqvist, P.-A., Müller, M.D. and Buser, H.-R. (2004) 'Occurrence of methyl triclosan, a transformation product of the bactericide triclosan, in fish from various lakes in Switzerland', *Environmental science & technology*, 38(2), pp. 390-395.

Bamford, H.A., Poster, D.L. and Baker, J.E. (1999) 'Temperature dependence of Henry's law constants of thirteen polycyclic aromatic hydrocarbons between 4 C and 31 C', *Environmental Toxicology and Chemistry*, 18(9), pp. 1905-1912.

Barbosa, M.O., Moreira, N.F.F., Ribeiro, A.R., Pereira, M.F.R. and Silva, A.M.T. (2016) 'Occurrence and removal of organic micropollutants: an overview of the watch list of EU Decision 2015/495', *Water research*, 94, pp. 257-279.

Batt, A.L., Kim, S. and Aga, D.S. (2006) 'Enhanced biodegradation of iopromide and trimethoprim in nitrifying activated sludge', *Environmental science & technology*, 40(23), pp. 7367-7373.

Baynes, A., Green, C., Nicol, E., Beresford, N., Kanda, R., Henshaw, A., Churchley, J. and Jobling, S. (2012) 'Additional treatment of wastewater reduces endocrine disruption in wild fish--a comparative study of tertiary and advanced treatments', *Environ Sci Technol*, 46(10), pp. 5565-73.

Behera, S.K., Kim, H.W., Oh, J.-E. and Park, H.-S. (2011) 'Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea', *Science of the Total Environment*, 409(20), pp. 4351-4360.

Bennett, A.F. (1984) 'Thermal dependence of muscle function', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 247(2), pp. R217-R229.

Bertilsson, S. and Widenfalk, A. (2002) 'Photochemical degradation of PAHs in freshwaters and their impact on bacterial growth–influence of water chemistry', *Hydrobiologia*, 469(1), pp. 23-32.

Bester, K. (2005) 'Fate of triclosan and triclosan-methyl in sewage treatmentplants and surface waters', *Archives of Environmental Contamination and Toxicology*, 49(1), pp. 9-17.

Biotage (2016) *Extraction of Polyaromatic Hydrocarbons (PAHs) from Natural Waters Using ISOLUTE PAH Solid Phase Extraction Columns* (plication Note IST1025A.V.1). [Online]. Available at: http://www.biotage.com/literature/download/ist1025a.v.1__extraction_of_pahs_from_natural_waters.pdf?ref=http%3A//www.biotage.com/search%3Fq%3DPAH%2520application%2520note (Accessed: 14/08/2017).

Brasil Bernardelli, J.K., Liz, M.V., Belli, T.J., Lobo-Recio, M.A. and Lapolli, F.R. (2015) 'Removal of estrogens by activated sludge under different conditions using batch experiments', *Brazilian Journal of Chemical Engineering*, 32(2), pp. 421-432.

Brum, D.M. and Netto, A.D.P. (2009) 'Polycyclic aromatic hydrocarbons in Tripuí River, Ouro Preto, MG, Brazil', *Journal of Hazardous Materials*, 165(1), pp. 447-453.

Buntner, D., Sánchez, A.S. and Garrido, J.M. (2011) 'Three stages MBR (methanogenic, aerobic biofilm and membrane filtration) for the treatment of low-strength wastewaters', *Water Science and Technology*, 64(2), pp. 397-402.

Busetti, F., Heitz, A., Cuomo, M., Badoer, S. and Traverso, P. (2006) 'Determination of sixteen polycyclic aromatic hydrocarbons in aqueous and solid samples from an Italian wastewater treatment plant', *Journal of chromatography A*, 1102(1-2), pp. 104-115.

Buth, J.M., Steen, P.O., Sueper, C., Blumentritt, D., Vikesland, P.J., Arnold, W.A. and McNeill, K. (2010) 'Dioxin photoproducts of triclosan and its chlorinated derivatives in sediment cores', *Environmental science & technology*, 44(12), pp. 4545-4551.

Caldwell, D.J., Mastrocco, F., Anderson, P.D., Länge, R. and Sumpter, J.P. (2012) 'Predicted‐no‐effect concentrations for the steroid estrogens estrone, 17β‐estradiol, estriol, and 17α‐ethinylestradiol', *Environmental toxicology and chemistry*, 31(6), pp. 1396-1406.

Cao, Z., Wang, Y., Ma, Y., Xu, Z., Shi, G., Zhuang, Y. and Zhu, T. (2005) 'Occurrence and distribution of polycyclic aromatic hydrocarbons in reclaimed water and surface water of Tianjin, China', *Journal of hazardous materials*, 122(1), pp. 51-59.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K. and Gordon, J.I. (2010) 'QIIME allows analysis of high-throughput community sequencing data', *Nature methods*, 7(5), pp. 335-336.

Cea-Barcia, G., Carrère, H., Steyer, J.P. and Patureau, D. (2013) 'Evidence for PAH removal coupled to the first steps of anaerobic digestion in sewage sludge', *International Journal of Chemical Engineering*, 2013.

Cha, J. and Cupples, A.M. (2010) 'Triclocarban and triclosan biodegradation at field concentrations and the resulting leaching potentials in three agricultural soils', *Chemosphere*, 81(4), pp. 494-499.

Chen, X., Nielsen, J.L., Furgal, K., Liu, Y., Lolas, I.B. and Bester, K. (2011) 'Biodegradation of triclosan and formation of methyl-triclosan in activated sludge under aerobic conditions', *Chemosphere*, 84(4), pp. 452-456.

Chen, Z., Song, Q., Cao, G. and Chen, Y. (2008) 'Photolytic degradation of triclosan in the presence of surfactants', *Chemical Papers*, 62(6), pp. 608-615.

Churchill, P.F., Morgan, A.C. and Kitchens, E. (2008) 'Characterization of a pyrene-degrading Mycobacterium sp. strain CH-2', *Journal of Environmental Science and Health Part B*, 43(8), pp. 698-706.

Clarke, B.O., Porter, N.A., Symons, R.K., Marriott, P.J., Stevenson, G.J. and Blackbeard, J.R. (2010) 'Investigating the distribution of polybrominated diphenyl ethers through an Australian wastewater treatment plant', *Science of the Total Environment*, 408(7), pp. 1604-1611.

Clarke, K.R. and Warwick, R.M. (2001) 'A further biodiversity index applicable to species lists: variation in taxonomic distinctness', *Marine ecology Progress series*, 216, pp. 265-278.

Clesceri, L.S.G., Eaton, A.E., Rice, A.D., Franson, E.W. and Mary Ann, H. (2005) *Standard methods for the examination of water and wastewater* (0875530478).

Coello-Garcia (2018) *Investigating the removal of estrogens in UK activated sludge plants*. Newcastle University.

Coleman, H.M., Le-Minh, N., Khan, S.J., Short, M.D., Chernicharo, C. and Stuetz, R.M. (2010) 'Fate and levels of steroid oestrogens and androgens in waste stabilisation ponds: quantification by liquid chromatography-tandem mass spectrometry', *Water Sci Technol*, 61(3), pp. 677-84.

Coleman, H.M., Routledge, E.J., Sumpter, J.P., Eggins, B.R. and Byrne, J.A. (2004) 'Rapid loss of estrogenicity of steroid estrogens by UVA photolysis and photocatalysis over an immobilised titanium dioxide catalyst', *Water research*, 38(14), pp. 3233-3240.

Combalbert, S. and Hernandez-Raquet, G. (2010) 'Occurrence, fate, and biodegradation of estrogens in sewage and manure', *Applied microbiology and biotechnology*, 86(6), pp. 1671-1692.

Cosmetics-europe (2017) *Cosmetics usage Europe*. Available at: https://www.cosmeticseurope.eu/cosmetic-products/ (Accessed: 23/02/2018).

Covaci, A., Dirtu, A.C., Voorspoels, S., Roosens, L. and Lepom, P. (2010) 'Sample preparation and chromatographic methods applied to congener-specific analysis of polybrominated diphenyl ethers', in *Brominated Flame Retardants*. Springer, pp. 55-94.

Cristale, J., Katsoyiannis, A., Sweetman, A.J., Jones, K.C. and Lacorte, S. (2013) 'Occurrence and risk assessment of organophosphorus and brominated flame retardants in the River Aire (UK)', *Environmental pollution*, 179, pp. 194-200.

Cristale, J. and Lacorte, S. (2015) 'PBDEs versus NBFR in wastewater treatment plants: occurrence and partitioning in water and sludge', *AIMS Environmental Science*, 2(3), pp. 533-546.

Cristale, J., Quintana, J., Chaler, R., Ventura, F. and Lacorte, S. (2012) 'Gas chromatography/mass spectrometry comprehensive analysis of organophosphorus, brominated flame retardants, by-products and formulation intermediates in water', *Journal of Chromatography A*, 1241, pp. 1-12.

Czajka, C.P. and Londry, K.L. (2006) 'Anaerobic biotransformation of estrogens', *Sci Total Environ*, 367(2-3), pp. 932-41.

Daso, A.P., Fatoki, O.S., Odendaal, J.P. and Olujimi, O.O. (2012) 'Occurrence of selected polybrominated diphenyl ethers and 2, 2′ , 4, 4′ , 5, 5′ -hexabromobiphenyl (BB-153) in sewage sludge and effluent samples of a wastewater-treatment plant in Cape Town, South Africa', *Archives of environmental contamination and toxicology*, 62(3), pp. 391-402.

Davies-Colley, R.J., Donnison, A.M., Speed, D.J., Ross, C.M. and Nagels, J.W.a. (1999) 'Inactivation of faecal indicator micro-organisms in waste stabilisation ponds: interactions of environmental factors with sunlight', *Water Research*, 33(5), pp. 1220-1230.

Davis, E.F. and Stapleton, H.M. (2009) 'Photodegradation pathways of nonabrominated diphenyl ethers, 2-ethylhexyltetrabromobenzoate and di (2-ethylhexyl) tetrabromophthalate: identifying potential markers of photodegradation', *Environmental science & technology*, 43(15), pp. 5739-5746.

De Maagd, P.G.J., Ten Hulscher, D.T.E.M., Van den Heuvel, H., Opperhuizen, A. and Sijm, D.T.H.M. (1998) 'Physicochemical properties of polycyclic aromatic hydrocarbons: Aqueous solubilities, n-octanol/water partition coefficients, and Henry's law constants', *Environmental Toxicology and Chemistry*, 17(2), pp. 251-257.

De Wit, C.A. (2002) 'An overview of brominated flame retardants in the environment', *Chemosphere*, 46(5), pp. 583-624.

Dean‐Ross, D., Moody, J. and Cerniglia, C.E. (2002) 'Utilization of mixtures of polycyclic aromatic hydrocarbons by bacteria isolated from contaminated sediment', *FEMS microbiology ecology*, 41(1), pp. 1-7.

Deblonde, T., Cossu-Leguille, C. and Hartemann, P. (2011) 'Emerging pollutants in wastewater: a review of the literature', *International journal of hygiene and environmental health*, 214(6), pp. 442-448.

Deng, D., Chen, H. and Tam, N.F.Y. (2015) 'Temporal and spatial contamination of polybrominated diphenyl ethers (PBDEs) in wastewater treatment plants in Hong Kong', *Science of the Total Environment*, 502, pp. 133-142.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P. and Andersen, G.L. (2006) 'Greengenes, a chimera-checked 16S rRNA

gene database and workbench compatible with ARB', *Applied and environmental microbiology*, 72(7), pp. 5069-5072.

Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T. and Gore, A.C. (2009) 'Endocrine-disrupting chemicals: an Endocrine Society scientific statement', *Endocrine reviews*, 30(4), pp. 293-342.

Dolan, J.W. (2009) *Calibration Curves , Part 1: To b or Not to b*. Available at: http://www.chromatographyonline.com/calibration-curves-part-i-b-or-not-b?id=&sk=&date=&pageID=5 (Accessed: 15 May, 2015).

Dytczak, M.A., Londry, K.L. and Oleszkiewicz, J.A. (2008) 'Biotransformation of estrogens in nitrifying activated sludge under aerobic and alternating anoxic/aerobic conditions', *Water Environment Research*, 80(1), pp. 47-52.

Ebele, A.J., Abdallah, M.A.-E. and Harrad, S. (2017) 'Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment', *Emerging Contaminants*, 3(1), pp. 1-16.

EC (1994) *PRIORITY SUBSTANCES LIST ASSESSMENT REPORT; POLYCYCLIC AROMATIC HYDROCARBONS*. Ottawa: Canadian Environmental Protection Act.

EC (2006) *Ecological Screening Assessment Report on Polybrominated Diphenyl Ethers (PBDEs); Canadian Environmental Protection Act, 1999*. Canada: Environment Canada.

ECHA (2017) *ECHA chemicals* Available at: https://echa.europa.eu/information-on-chemicals.

Esperanza, M., Suidan, M.T., Marfil-Vega, R., Gonzalez, C., Sorial, G.A., McCauley, P. and Brenner, R. (2007) 'Fate of sex hormones in two pilot-scale municipal wastewater treatment plants: Conventional treatment', *Chemosphere*, 66(8), pp. 1535-1544.

EU (2012) *Proposal for a revised directive of the European Parliarment and of the Council on Priority Substances in the field of water quality, MEMO/12/59, 31 January 2012, Brussels*.

EU (2013a) *DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy*. [Online]. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013L0039&from=EN.

EU, C. (2013b) 'Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy', *Off. J. Eur. Union*, 226, pp. 1-17.

Expertise (2015) *PCB Removal Technologies*. Available at: http://www.expertise-limited.co.uk/PolyCHlorinatedBiphenylsWaterTreatment.htm (Accessed: 27/02/2018).

Fatone, F., Di Fabio, S., Bolzonella, D. and Cecchi, F. (2011) 'Fate of aromatic hydrocarbons in Italian municipal wastewater systems: An overview of wastewater treatment using conventional activated-sludge processes (CASP) and membrane bioreactors (MBRs)', *Water Research*, 45(1), pp. 93-104.

Feng, Y., Zhang, P., Zhang, Z., Shi, J., Jiao, Z. and Shao, B. (2016) 'Endocrine disrupting effects of triclosan on the placenta in pregnant rats', *PloS one*, 11(5), p. e0154758.

Fiss, E.M., Rule, K.L. and Vikesland, P.J. (2007) 'Formation of chloroform and other chlorinated byproducts by chlorination of triclosan-containing antibacterial products', *Environmental science & technology*, 41(7), pp. 2387-2394.

Foght, J. (2008) 'Anaerobic biodegradation of aromatic hydrocarbons: pathways and prospects', *Journal of Molecular Microbiology and Biotechnology*, 15(2-3), pp. 93-120.

Fontana, A.R., Silva, M.F., Martínez, L.D., Wuilloud, R.G. and Altamirano, J.C. (2009) 'Determination of polybrominated diphenyl ethers in water and soil samples by cloud point extraction-ultrasound-assisted back-extraction-gas chromatography–mass spectrometry', *Journal of Chromatography A*, 1216(20), pp. 4339-4346.

Froehner, S., Piccioni, W., Machado, K.S. and Aisse, M.M. (2010) 'Removal Capacity of Caffeine, Hormones, and Bisphenol by Aerobic and Anaerobic Sewage Treatment', *Water, Air, & Soil Pollution*, 216(1-4), pp. 463-471.

Gabet-Giraud, V., Miège, C., Choubert, J.M., Ruel, S.M. and Coquery, M. (2010) 'Occurrence and removal of estrogens and beta blockers by various processes in wastewater treatment plants', *Science of the total environment*, 408(19), pp. 4257-4269.

Gallego, S., Vila, J., Tauler, M., Nieto, J.M., Breugelmans, P., Springael, D. and Grifoll, M. (2014) 'Community structure and PAH ring-hydroxylating dioxygenase genes of a marine pyrene-degrading microbial consortium', *Biodegradation*, 25(4), pp. 543-556.

Garcia, I.M., Mokosch, M., Soares, A., Pidou, M. and Jefferson, B. (2013) 'Impact on reactor configuration on the performance of anaerobic MBRs: treatment of settled sewage in temperate climates', *Water research*, 47(14), pp. 4853-4860.

Gardner, M., Comber, S., Scrimshaw, M.D., Cartmell, E., Lester, J. and Ellor, B. (2012) 'The significance of hazardous chemicals in wastewater treatment works effluents', *Science of the Total Environment*, 437, pp. 363-372.

Gaulke, L.S., Strand, S.E., Kalhorn, T.F. and Stensel, H.D. (2008) '17 alpha-ethinylestradiol Transformation via Abiotic Nitration in the Presence of Ammonia Oxidizing Bacteria', *Environmental Science & Technology*, 42(20), pp. 7622-7627.

Gee, D., Grandjean, P., Hansen, S.F., Van denHove, S., MacGarvin, M., Martin, J., Nielsen, G., Quist, D. and Stanners, D. (2013) 'Late lessons from early warnings: science, precaution, innovation', *European Environment Agency. Copenhagen, Denmark*.

Gerecke, A.C., Hartmann, P.C., Heeb, N.V., Kohler, H.-P.E., Giger, W., Schmid, P., Zennegg, M. and Kohler, M. (2005) 'Anaerobic degradation of decabromodiphenyl ether', *Environmental science & technology*, 39(4), pp. 1078-1083.

Ghosal, D., Ghosh, S., Dutta, T.K. and Ahn, Y. (2016) 'Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review', *Frontiers in microbiology*, 7.

Gilbert, N. (2012) 'Drug-pollution law all washed up', *Nature*, 491(7425), pp. 503-504.

Gomez, E., Wang, X., Dagnino, S., Leclercq, M., Escande, A., Casellas, C., Picot, B. and Fenet, H. (2007) 'Fate of endocrine disrupters in waste stabilization pond systems', *Water Science & Technology*, 55(11), p. 157.

González, G.M.E. (2006) *Enzymatic degradation of polycyclic aromatic hydrocarbons (PAHs) by manganese peroxidase in reactors containing organic solvents*. Univ Santiago de Compostela.

Gorga, M., Martinez, E., Ginebreda, A., Eljarrat, E. and Barcelo, D. (2013) 'Determination of PBDEs, HBB, PBEB, DBDPE, HBCD, TBBPA and related compounds in sewage sludge from Catalonia (Spain)', *Science of the Total Environment*, 444, pp. 51-59.

Gray, A. (2009) *http://www.globalurban.org/Belo_Horizonte_MES.pdf*. Available at: http://www.globalurban.org/Belo_Horizonte_MES.pdf (Accessed: 12/02/2018).

Haarmeyer, D. (2011) 'A Fresh Look at US Water and Wastewater Infrastructure: The Commercial and Environmentally Sustainable Path Forward', *Journal of Applied Corporate Finance*, 23(3), pp. 41-52.

Haiyan, R., Shulan, J., ud din Ahmad, N., Dao, W. and Chengwu, C. (2007) 'Degradation characteristics and metabolic pathway of 17alpha-ethynylestradiol by Sphingobacterium sp. JCR5', *Chemosphere*, 66(2), pp. 340-6.

Hale, R.C., Alaee, M., Manchester-Neesvig, J.B., Stapleton, H.M. and Ikonomou, M.G. (2003) 'Polybrominated diphenyl ether flame retardants in the North American environment', *Environment International*, 29(6), pp. 771-779.

Haritash, A.K. and Kaushik, C.P. (2009) 'Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review', *Journal of hazardous materials*, 169(1), pp. 1-15.

Harremoës, P., Gee, D., MacGarvin, M., Stirling, A., Keys, J., Wynne, B. and Vaz, S.G. (2001) *Late lessons from early warnings: the precautionary principle 1896-2000*. Citeseer.

He, J., Robrock, K.R. and Alvarez-Cohen, L. (2006) 'Microbial reductive debromination of polybrominated diphenyl ethers (PBDEs)', *Environmental Science & Technology*, 40(14), pp. 4429-4434.

Heidler, J. and Halden, R.U. (2007) 'Mass balance assessment of triclosan removal during conventional sewage treatment', *Chemosphere*, 66(2), pp. 362-369.

Heidrich, E.S., Curtis, T.P. and Dolfing, J. (2010) 'Determination of the internal chemical energy of wastewater', *Environmental Science & Technology*, 45(2), pp. 827-832.

Helbling, D.E., Hollender, J., Kohler, H.-P.E., Singer, H. and Fenner, K. (2010) 'High-throughput identification of microbial transformation products of organic micropollutants', *Environmental science & technology*, 44(17), pp. 6621-6627.

Helbling, D.E., Johnson, D.R., Honti, M. and Fenner, K. (2012) 'Micropollutant biotransformation kinetics associate with WWTP process parameters and microbial community characteristics', *Environmental science & technology*, 46(19), pp. 10579-10588.

Howe, A. (2009) *Renewable energy potential for the water industry*. Environment Agency.

Huang, L., Hwang, A. and Phillips, J. (2011) 'Effect of temperature on microbial growth rate–mathematical analysis: the Arrhenius and Eyring–Polanyi connections', *Journal of food science*, 76(8).

Hutzinger, O., Beek, B. and Metzler, M. (2013) *The handbook of environmental chemistry*. Springer.

Hutzinger, O., Beek, B. and Metzler, M. (2013) *The handbook of environmental chemistry*. Springer.

Indexmundi (2017) *Brazil Age Structure*. Available at: https://www.indexmundi.com/brazil/age_structure.html.

Ishibashi, H., Matsumura, N., Hirano, M., Matsuoka, M., Shiratsuchi, H., Ishibashi, Y., Takao, Y. and Arizono, K. (2004) 'Effects of triclosan on the early life stages and reproduction of medaka Oryzias latipes and induction of hepatic vitellogenin', *Aquatic Toxicology*, 67(2), pp. 167-179.

Jiao, S., Chen, W., Wang, E., Wang, J., Liu, Z., Li, Y. and Wei, G. (2016) 'Microbial succession in response to pollutants in batch-enrichment culture', *Scientific reports*, 6, p. 21791.

Johnson, A.C. and Williams, R.J. (2004) 'A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works', *Environmental science & technology*, 38(13), pp. 3649-3658.

Jones, L., Kinsella, B., Furey, A. and Regan, F. (2012) 'Monitoring the occurrence of PAHs in Irish wastewater effluent', *Journal of Environmental Monitoring*, 14(11), pp. 3009-3014.

Jones, M.D., Rodgers-Vieira, E.A., Hu, J. and Aitken, M.D. (2014) 'Association of growth substrates and bacterial genera with benzo [a] pyrene mineralization in contaminated soil', *Environmental engineering science*, 31(12), pp. 689-697.

Joss, A., Andersen, H., Ternes, T., Richle, P.R. and Siegrist, H. (2004) 'Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: Consequences for plant optimization', *Environmental Science & Technology*, 38(11), pp. 3047-3055.

Kantiani, L., Farré, M., Asperger, D., Rubio, F., González, S., de Alda, M.J.L., Petrović, M., Shelver, W.L. and Barceló, D. (2008) 'Triclosan and methyl-triclosan monitoring study in the northeast of Spain using a magnetic particle enzyme immunoassay and confirmatory analysis by gas chromatography–mass spectrometry', *Journal of hydrology*, 361(1), pp. 1-9.

Karathanasis, A.D. and Johnson, C.M. (2003) 'Metal removal potential by three aquatic plants in an acid mine drainage wetland', *Mine Water and the Environment*, 22(1), pp. 22-30.

Kim, M., Guerra, P., Theocharides, M., Barclay, K., Smyth, S.A. and Alaee, M. (2013) 'Parameters affecting the occurrence and removal of polybrominated diphenyl ethers in twenty Canadian wastewater treatment plants', *water research*, 47(7), pp. 2213-2221.

Kim, S.D., Cho, J., Kim, I.S., Vanderford, B.J. and Snyder, S.A. (2007) 'Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters', *Water research*, 41(5), pp. 1013-1021.

Kim, Y.-H., Freeman, J.P., Moody, J.D., Engesser, K.-H. and Cerniglia, C.E. (2005) 'Effects of pH on the degradation of phenanthrene and pyrene by Mycobacterium vanbaalenii PYR-1', *Applied microbiology and biotechnology*, 67(2), pp. 275-285.

Kochany, J. and Maguire, R.J. (1994) 'Abiotic transformations of polynuclear aromatic hydrocarbons and polynuclear aromatic nitrogen heterocycles in aquatic environments', *Science of the total environment*, 144(1-3), pp. 17-31.

Kookana, R.S., Ying, G.G. and Waller, N.J. (2011) 'Triclosan: its occurrence, fate and effects in the Australian environment', *Water Science and Technology*, 63(4), pp. 598-604.

Koutsoumanis, K.P., Taoukis, P.S., Drosinos, E.H. and Nychas, G.-J.E. (2000) 'Applicability of an Arrhenius model for the combined effect of temperature and CO2 packaging on the spoilage microflora of fish', *Applied and Environmental Microbiology*, 66(8), pp. 3528-3534.

Kumar, K.S., Priya, S.M., Peck, A.M. and Sajwan, K.S. (2010) 'Mass loadings of triclosan and triclocarbon from four wastewater treatment plants to three rivers and landfill in Savannah, Georgia, USA', *Archives of environmental contamination and toxicology*, 58(2), pp. 275-285.

Kummer, V., Mašková, J., Zralý, Z., Neča, J., Šimečková, P., Vondráček, J. and Machala, M. (2008) 'Estrogenic activity of environmental polycyclic aromatic hydrocarbons in uterus of immature Wistar rats', *Toxicology letters*, 180(3), pp. 212-221.

Labadie, P., Tlili, K., Alliot, F., Bourges, C., Desportes, A. and Chevreuil, M. (2010) 'Development of analytical procedures for trace-level determination of polybrominated diphenyl ethers and tetrabromobisphenol A in river water and sediment', *Analytical and bioanalytical chemistry*, 396(2), pp. 865-875.

Laboratories, W. (2009) *2,2',3,3',4,5,5',6,6'-Nonabromo-4'-chlorodiphenyl ether (4PC-BDE-208)*.

Landrigan, P.J., Fuller, R., Acosta, N.J.R., Adeyi, O., Arnold, R., Baldé, A.B., Bertollini, R., Bose-O'Reilly, S., Boufford, J.I. and Breysse, P.N. (2017) 'The Lancet Commission on pollution and health', *The Lancet*.

Lee, D.G. and Chu, K.-H. (2013) 'Effects of growth substrate on triclosan biodegradation potential of oxygenase-expressing bacteria', *Chemosphere*, 93(9), pp. 1904-1911.

Lee, H.-B., Sarafin, K., Peart, T.E. and Svoboda, M.L. (2003) 'Acidic pharmaceuticals in sewage-Methodology, stability test, occurrence, and removal from Ontario samples', *Water Quality Research Journal of Canada*, 38(4), pp. 667-682.

Lee, S., Song, G.J., Kannan, K. and Moon, H.B. (2014) 'Occurrence of PBDEs and other alternative brominated flame retardants in sludge from wastewater treatment plants in Korea', *Sci Total Environ*, 470-471, pp. 1422-9.

Lei, A.-P., Hu, Z.-L., Wong, Y.-S. and Tam, N.F.-Y. (2007) 'Removal of fluoranthene and pyrene by different microalgal species', *Bioresource technology*, 98(2), pp. 273-280.

Leusch, F.D.L., Chapman, H.F., van den Heuvel, M.R., Tan, B.L.L., Gooneratne, S.R. and Tremblay, L.A. (2006) 'Bioassay-derived androgenic and estrogenic activity in municipal sewage in Australia and New Zealand', *Ecotoxicology and Environmental Safety*, 65(3), pp. 403-411.

Levenspiel, O. (1999) 'Chemical reaction engineering', *Industrial & engineering chemistry research*, 38(11), pp. 4140-4143.

Li, M.K.-Y., Lei, N.-Y., Gong, C., Yu, Y., Lam, K.-H., Lam, M.H.-W., Yu, H. and Lam, P.K.-S. (2009) 'An organically modified silicate molecularly imprinted solid-phase microextraction device for the determination of polybrominated diphenyl ethers', *Analytica chimica acta*, 633(2), pp. 197-203.

Li, W.-W., Yu, H.-Q. and Rittmann, B.E. (2015) 'Reuse water pollutants', *Nature*, 528(7580), p. 29.

Lindström, A., Buerge, I.J., Poiger, T., Bergqvist, P.-A., Müller, M.D. and Buser, H.-R. (2002) 'Occurrence and environmental behavior of the bactericide triclosan and its methyl derivative in surface waters and in wastewater', *Environmental science & technology*, 36(11), pp. 2322-2329.

Liu, H., Ramnarayanan, R. and Logan, B.E. (2004) 'Production of electricity during wastewater treatment using a single chamber microbial fuel cell', *Environmental science & technology*, 38(7), pp. 2281-2285.

Liu, J.J., Wang, X.C. and Fan, B. (2011) 'Characteristics of PAHs adsorption on inorganic particles and activated sludge in domestic wastewater treatment', *Bioresource technology*, 102(9), pp. 5305-5311.

Liu, Z.-h., Kanjo, Y. and Mizutani, S. (2010) 'A review of phytoestrogens: Their occurrence and fate in the environment', *Water Research*, 44(2), pp. 567-577.

Liu, Z.-h., Lu, G.-n., Yin, H., Dang, Z. and Rittmann, B. (2015) 'Removal of natural estrogens and their conjugates in municipal wastewater treatment plants: a critical review', *Environmental science & technology*, 49(9), pp. 5288-5300.

Liu, Z.H., Kanjo, Y. and Mizutani, S. (2009) 'Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment - physical means, biodegradation, and chemical advanced oxidation: a review', *Sci Total Environ*, 407(2), pp. 731-48.

Lofrano, G. (2012) *Emerging compounds removal from wastewater: Natural and Solar Based Treatments*. Springer Science & Business Media.

Logan, B.E. (2005) 'Simultaneous wastewater treatment and biological electricity generation', *Water Science and Technology*, 52(1-2), pp. 31-37.

Lozano, N., Rice, C.P., Ramirez, M. and Torrents, A. (2013) 'Fate of Triclocarban, Triclosan and Methyltriclosan during wastewater and biosolids treatment processes', *Water Research*, 47(13), pp. 4519-4527.

Lucas, S.D. and Jones, D.L. (2006) 'Biodegradation of estrone and 17 β-estradiol in grassland soils amended with animal wastes', *Soil Biology and Biochemistry*, 38(9), pp. 2803-2815.

Luo, Y.L., Guo, W.S., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S. and Wang, X.C.C. (2014) 'A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment', *Science of the Total Environment*, 473, pp. 619-641.

Mackay, D., Shiu, W.-Y., Ma, K.-C. and Lee, S.C. (2006) *Handbook of physical-chemical properties and environmental fate for organic chemicals*. CRC press.

Mai, B., Chen, S., Chen, S., Luo, X., Chen, L., Chen, L., Yang, Q., Sheng, G., Peng, P. and Fu, J. (2005) 'Distribution of polybrominated diphenyl ethers in sediments of the Pearl River Delta and adjacent South China Sea', *Environmental Science & Technology*, 39(10), pp. 3521-3527.

Manoli, E. and Samara, C. (2008) 'The removal of Polycyclic Aromatic Hydrocarbons in the wastewater treatment process: Experimental calculations and model predictions', *Environmental Pollution*, 151(3), pp. 477-485.

Martin, T.J., Snape, J.R., Bartram, A., Robson, A., Acharya, K. and Davenport, R.J. (2017) 'Environmentally relevant inoculum concentrations improve the reliability of persistent assessments in biodegradation screening tests', *Environmental science & technology*, 51(5), pp. 3065-3073.

McCarty, P.L., Bae, J. and Kim, J. (2011) 'Domestic wastewater treatment as a net energy producer–can this be achieved?'. ACS Publications.

Moja, S.J. and Mtunzi, F. (2013) 'Application of solid phase extraction (SPE) method in determining polycyclic aromatic hydrocarbons (PAHs) in river water samples', *Journal of Environmental Chemistry and Ecotoxicology*, 5(11), pp. 278-283.

Moody, J.D., Freeman, J.P., Doerge, D.R. and Cerniglia, C.E. (2001) 'Degradation of phenanthrene and anthracene by cell suspensions of Mycobacterium sp. strain PYR-1', *Applied and environmental microbiology*, 67(4), pp. 1476-1483.

Nácher-Mestre, J., Serrano, R., Hernández, F., Benedito-Palos, L. and Pérez-Sánchez, J. (2010) 'Gas chromatography–mass spectrometric determination of polybrominated diphenyl ethers in complex fatty matrices from aquaculture activities', *Analytica chimica acta*, 664(2), pp. 190-198.

Nie, Y., Qiang, Z., Zhang, H. and Ben, W. (2012) 'Fate and seasonal variation of endocrine-disrupting chemicals in a sewage treatment plant with A/A/O process', *Separation and purification technology*, 84, pp. 9-15.

North, K.D. (2004) 'Tracking polybrominated diphenyl ether releases in a wastewater treatment plant effluent, Palo Alto, California', *Environmental Science & Technology*, 38(17), pp. 4484-4488.

Novak, J.T., Goldsmith, C.D., Benoit, R.E. and O'Brien, J.H. (1985) 'Biodegradation of methanol and tertiary butyl alcohol in subsurface systems', *Water Science and Technology*, 17(9), pp. 71-85.

Oberoi, A.S., Philip, L. and Bhallamudi, S.M. (2015) 'Biodegradation of Various Aromatic Compounds by Enriched Bacterial Cultures: Part A–Monocyclic and Polycyclic Aromatic Hydrocarbons', *Applied biochemistry and biotechnology*, 176(7), pp. 1870-1888.

Oecd (1994) *OECD Guidelines for the Testing of Chemicals*. Organization for Economic.

Ogunyoku, T.A. and Young, T.M. (2014) 'Removal of triclocarban and triclosan during municipal biosolid production', *Water Environment Research*, 86(3), pp. 197-203.

Pan, Y., Tsang, D.C.W., Wang, Y., Li, Y. and Yang, X. (2016) 'The photodegradation of polybrominated diphenyl ethers (PBDEs) in various environmental matrices: Kinetics and mechanisms', *Chemical Engineering Journal*, 297, pp. 74-96.

Parada, A.E., Needham, D.M. and Fuhrman, J.A. (2016) 'Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples', *Environmental microbiology*, 18(5), pp. 1403-1414.

Parks, D.H., Tyson, G.W., Hugenholtz, P. and Beiko, R.G. (2014) 'STAMP: statistical analysis of taxonomic and functional profiles', *Bioinformatics*, 30(21), pp. 3123-3124.

Peng, R.-H., Xiong, A.-S., Xue, Y., Fu, X.-Y., Gao, F., Zhao, W., Tian, Y.-S. and Yao, Q.-H. (2008) 'Microbial biodegradation of polyaromatic hydrocarbons', *FEMS microbiology reviews*, 32(6), pp. 927-955.

Peng, X.Z., Tang, C.M., Yu, Y.Y., Tan, J.H., Huang, Q.X., Wu, J.P., Chen, S.J. and Mai, B.X. (2009) 'Concentrations, transport, fate, and releases of polybrominated diphenyl ethers in sewage treatment plants in the Pearl River Delta, South China', *Environment International*, 35(2), pp. 303-309.

Pessoa, G.P., de Souza, N.C., Vidal, C.B., Alves, J.A., Firmino, P.I., Nascimento, R.F. and Dos Santos, A.B. (2014) 'Occurrence and removal of estrogens in Brazilian wastewater treatment plants', *Sci Total Environ*, 490C, pp. 288-295.

Petrie, B., McAdam, E.J., Hassard, F., Stephenson, T., Lester, J.N. and Cartmell, E. (2014) 'Diagnostic investigation of steroid estrogen removal by activated sludge at varying solids retention time', *Chemosphere*, 113, pp. 101-108.

Petropoulos, E., Dolfing, J. and Curtis, T.P. (2017a) *Frontiers International Conference on Wastewater Treatment and Modelling*. Springer.

Petropoulos, E., Dolfing, J., Davenport, R.J., Bowen, E.J. and Curtis, T.P. (2017b) 'Developing cold-adapted biomass for the anaerobic treatment of domestic wastewater at low temperatures (4, 8 and 15 C) with inocula from cold environments', *Water research*, 112, pp. 100-109.

Pitarch, E., Medina, C., Portolés, T., López, F.J. and Hernández, F. (2007) 'Determination of priority organic micro-pollutants in water by gas chromatography coupled to triple quadrupole mass spectrometry', *Analytica chimica acta*, 583(2), pp. 246-258.

Pitter, P. and Chudoba, J. (1990) *Biodegradability of organic substance in the aquatic environment*.

Price, F.T., Brix, K.V. and Lane, N.K. (2000). ASTM.

Qi, W., Liu, H., Pernet-Coudrier, B. and Qu, J. (2013) 'Polycyclic aromatic hydrocarbons in wastewater, WWTPs effluents and in the recipient waters of Beijing, China', *Environmental Science and Pollution Research*, 20(6), pp. 4254-4260.

Qiao, M., Qi, W., Liu, H. and Qu, J. (2013) 'Simultaneous determination of typical substituted and parent polycyclic aromatic hydrocarbons in water and solid matrix by gas chromatography–mass spectrometry', *Journal of Chromatography A*, 1291, pp. 129-136.

Qiao, M., Qi, W.X., Liu, H.J. and Qu, J.H. (2014) 'Occurrence, behavior and removal of typical substituted and parent polycyclic aromatic hydrocarbons in a biological wastewater treatment plant', *Water Research*, 52, pp. 11-19.

Queiroz, F.B., Brandt, E.M., Aquino, S.F., Chernicharo, C.A. and Afonso, R.J. (2012) 'Occurrence of pharmaceuticals and endocrine disruptors in raw sewage and their behavior in UASB reactors operated at different hydraulic retention times', *Water Sci Technol*, 66(12), pp. 2562-9.

Racz, L. and Goel, R.K. (2010) 'Fate and removal of estrogens in municipal wastewater', *Journal of Environmental Monitoring*, 12(1), pp. 58-70.

Rahman, F., Langford, K.H., Scrimshaw, M.D. and Lester, J.N. (2001) 'Polybrominated diphenyl ether (PBDE) flame retardants', *Science of the Total Environment*, 275(1-3), pp. 1-17.

Ratola, N., Cincinelli, A., Alves, A. and Katsoyiannis, A. (2012) 'Occurrence of organic microcontaminants in the wastewater treatment process. A mini review', *Journal of Hazardous Materials*, 239, pp. 1-18.

Reeder, J. and Knight, R. (2010) 'Rapidly denoising pyrosequencing amplicon reads by exploiting rank-abundance distributions', *Nature methods*, 7(9), pp. 668-669.

Ren, Y.-X., Nakano, K., Nomura, M., Chiba, N. and Nishimura, O. (2007) 'Effects of bacterial activity on estrogen removal in nitrifying activated sludge', *Water Research*, 41(14), pp. 3089-3096.

Robrock, K.R., Coelhan, M., Sedlak, D.L. and Alvarez-Cohen, L. (2009) 'Aerobic biotransformation of polybrominated diphenyl ethers (PBDEs) by bacterial isolates', *Environmental science & technology*, 43(15), pp. 5705-5711.

Rogers, H.R. (1996) 'Sources, behaviour and fate of organic contaminants during sewage treatment and in sewage sludges', *Science of the total environment*, 185(1-3), pp. 3-26.

Roh, H., Subramanya, N., Zhao, F., Yu, C.-P., Sandt, J. and Chu, K.-H. (2009) 'Biodegradation potential of wastewater micropollutants by ammonia-oxidizing bacteria', *Chemosphere*, 77(8), pp. 1084-1089.

Rücker, C. and Kümmerer, K. (2012) 'Modeling and predicting aquatic aerobic biodegradation–a review from a user's perspective', *Green Chemistry*, 14(4), pp. 875-887.

Sabaliunas, D., Webb, S.F., Hauk, A., Jacob, M. and Eckhoff, W.S. (2003) 'Environmental fate of triclosan in the River Aire Basin, UK', *Water Research*, 37(13), pp. 3145-3154.

Saeed, T., Ali, L.N., Al-Bloushi, A., Al-Hashash, H., Al-Bahloul, M., Al-Khabbaz, A. and Al-Khayat, A. (2011) 'Effect of environmental factors on photodegradation of polycyclic aromatic hydrocarbons (PAHs) in the water-soluble fraction of Kuwait crude oil in seawater', *Marine environmental research*, 72(3), pp. 143-150.

Salgado, R., Noronha, J.P., Oehmen, A., Carvalho, G. and Reis, M.A. (2010) 'Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology', *Water Sci Technol*, 62(12), pp. 2862-71.

Samaras, V.G., Thomaidis, N.S., Stasinakis, A.S. and Lekkas, T.D. (2011) 'An analytical method for the simultaneous trace determination of acidic pharmaceuticals and phenolic endocrine disrupting chemicals in wastewater and sewage sludge by gas chromatography-mass spectrometry', *Analytical and bioanalytical chemistry*, 399(7), pp. 2549-2561.

Sánchez-Avila, J., Bonet, J., Velasco, G. and Lacorte, S. (2009) 'Determination and occurrence of phthalates, alkylphenols, bisphenol A, PBDEs, PCBs and PAHs in an industrial sewage grid discharging to a Municipal Wastewater Treatment Plant', *Science of the Total Environment*, 407(13), pp. 4157-4167.

Sanchez-Brunete, C., Miguel, E. and Tadeo, J.L. (2009) 'Determination of tetrabromobisphenol-A, tetrachlorobisphenol-A and bisphenol-A in soil by ultrasonic assisted extraction and gas chromatography-mass spectrometry', *Journal of Chromatography A*, 1216(29), pp. 5497-5503.

Sánchez-Brunete, C., Miguel, E. and Tadeo, J.L. (2007) 'Analysis of 27 polycyclic aromatic hydrocarbons by matrix solid-phase dispersion and isotope dilution gas chromatography-mass spectrometry in sewage sludge from the Spanish area of Madrid', *Journal of Chromatography A*, 1148(2), pp. 219-227.

Santos, M.M.d., Brehm, F.d.A., Filippe, T.C., Knapik, H.G. and Azevedo, J.C.R.d. (2016) 'Occurrence and risk assessment of parabens and triclosan in surface waters of southern Brazil: a problem of emerging compounds in an emerging country', *RBRH*, 21(3), pp. 603-617.

Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., Von Gunten, U. and Wehrli, B. (2006) 'The challenge of micropollutants in aquatic systems', *Science*, 313(5790), pp. 1072-1077.

Seo, J.-S., Keum, Y.-S. and Li, Q.X. (2009) 'Bacterial degradation of aromatic compounds', *International journal of environmental research and public health*, 6(1), pp. 278-309.

Shaw, A.K., Halpern, A.L., Beeson, K., Tran, B., Venter, J.C. and Martiny, J.B.H. (2008) 'It's all relative: ranking the diversity of aquatic bacterial communities', *Environmental microbiology*, 10(9), pp. 2200-2210.

Shi, J., Fujisawa, S., Nakai, S. and Hosomi, M. (2004) 'Biodegradation of natural and synthetic estrogens by nitrifying activated sludge and ammonia-oxidizing bacterium Nitrosomonas europaea', *Water Research*, 38(9), pp. 2323-2330.

Shiu, W.-Y. and Mackay, D. (1997) 'Henry's law constants of selected aromatic hydrocarbons, alcohols, and ketones', *Journal of Chemical & Engineering Data*, 42(1), pp. 27-30.

Siddiqi, M.A., Laessig, R.H. and Reed, K.D. (2003) 'Polybrominated diphenyl ethers (PBDEs): new pollutants–old diseases', *Clinical Medicine & Research*, 1(4), pp. 281-290.

Sigma (2011) *Derivatization Reagents For Selective Response and Detection in Complex Matrices* (T407138B). [Online]. Available at: http://www.sigmaaldrich.com/content/dam/sigma-aldrich/migrationresource4/Derivatization%20Rgts%20brochure.pdf.

Silva, C.P., Otero, M. and Esteves, V. (2012) 'Processes for the elimination of estrogenic steroid hormones from water: a review', *Environ Pollut*, 165, pp. 38-58.

Singer, H., Müller, S., Tixier, C. and Pillonel, L. (2002) 'Triclosan: occurrence and fate of a widely used biocide in the aquatic environment: field measurements in wastewater treatment plants, surface waters, and lake sediments', *Environmental science & technology*, 36(23), pp. 4998-5004.

Smith, A.L., Skerlos, S.J. and Raskin, L. (2015a) 'Anaerobic membrane bioreactor treatment of domestic wastewater at psychrophilic temperatures ranging from 15 C to 3 C', *Environmental Science: Water Research & Technology*, 1(1), pp. 56-64.

Smith, B.J.K., Boothe, M.A., Fiddler, B.A., Lozano, T.M., Rahi, R.K. and Krzmarzick, M.J. (2015b) 'Enumeration of organohalide respirers in municipal wastewater anaerobic digesters', *Microbiology insights*, 8, p. MBI. S31445.

Song, M., Chu, S.G., Letcher, R.J. and Seth, R. (2006) 'Fate, partitioning, and mass loading of polybrominated diphenyl ethers (PBDEs) during the treatment processing of municipal sewage', *Environmental Science & Technology*, 40(20), pp. 6241-6246.

Sornalingam, K., McDonagh, A. and Zhou, J.L. (2016) 'Photodegradation of estrogenic endocrine disrupting steroidal hormones in aqueous systems: progress and future challenges', *Science of the Total Environment*, 550, pp. 209-224.

Stapleton, H.M. (2006) 'Instrumental methods and challenges in quantifying polybrominated diphenyl ethers in environmental extracts: a review', *Analytical and bioanalytical chemistry*, 386(4), pp. 807-817.

Stapleton, R.D., Savage, D.C., Sayler, G.S. and Stacey, G. (1998) 'Biodegradation of aromatic hydrocarbons in an extremely acidic environment', *Applied and environmental microbiology*, 64(11), pp. 4180-4184.

Stenstrom, M.K., Cardinal, L. and Libra, J. (1989) 'Treatment of hazardous substances in wastewater treatment plants', *Environmental Progress & Sustainable Energy*, 8(2), pp. 107-112.

Stiborova, H., Vrkoslavova, J., Lovecka, P., Pulkrabova, J., Hradkova, P., Hajslova, J. and Demnerova, K. (2015) 'Aerobic biodegradation of selected polybrominated diphenyl ethers (PBDEs) in wastewater sewage sludge', *Chemosphere*, 118, pp. 315-321.

Suarez, S., Lema, J.M. and Omil, F. (2010) 'Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions', *Water Res*, 44(10), pp. 3214-24.

Tadkaew, N., Hai, F.I., McDonald, J.A., Khan, S.J. and Nghiem, L.D. (2011) 'Removal of trace organics by MBR treatment: the role of molecular properties', *Water research*, 45(8), pp. 2439-2451.

Tamura, I. and Yamamoto, H. (2012) 'Biodegradation and Photodegradation of Selected Antimicrobial/ Antifungal Agents in the Aquatic Environment and Their Sorption onto Sediments', *Journal of Environmental Chemistry*, 22(3), pp. 113-119.

Tchobanoglous, G., Stensel, D., Tsuchihashi, R., Burton, F.L., Mohammad, A.O., Bowden, G. and Pfrang, W. (2014) 'Wastewater Engineering: Treatment and Resource Recovery (Metcalf & Eddy, Inc'. McGraw-Hill, New York.

Ternes, T. and Joss, A. (2007) *Human pharmaceuticals, hormones and fragrances*. IWA publishing.

Ternes, T.A., Joss, A. and Siegrist, H. (2004) 'Peer reviewed: scrutinizing pharmaceuticals and personal care products in wastewater treatment'. ACS Publications.

Thomas, P.M. and Foster, G.D. (2005) 'Tracking acidic pharmaceuticals, caffeine, and triclosan through the wastewater treatment process', *Environmental Toxicology and Chemistry*, 24(1), pp. 25-30.

Thompson, A., Griffin, P., Stuetz, R. and Cartmell, E. (2005) 'The fate and removal of triclosan during wastewater treatment', *Water environment research*, 77(1), pp. 63-67.

Tittlemier, S.A., Halldorson, T., Stern, G.A. and Tomy, G.T. (2002) 'Vapor pressures, aqueous solubilities, and Henry's law constants of some brominated flame retardants', *Environmental Toxicology and Chemistry*, 21(9), pp. 1804-1810.

Tohidi, F. and Cai, Z. (2015) 'GC/MS analysis of triclosan and its degradation by-products in wastewater and sludge samples from different treatments', *Environmental Science and Pollution Research*, 22(15), pp. 11387-11400.

Trably, E., Patureau, D. and Delgenes, J.P. (2005) 'Polycyclic aromatic hydrocarbons (PAHs) removal during anaerobic and aerobic sludge treatments', in *Environmental Chemistry*. Springer, pp. 431-440.

Travel-Guide (2018) *Belo horizonte Travel Guide*. Available at: http://www.world66.com/southamerica/brazil/belohorizonte (Accessed: 10/01/2018).

UKTAG (2009) *Proposed EQS for Water Framework Directive Annex VIII substances: triclosan (For consultation)*. [Online]. Available at: https://www.wfduk.org/sites/default/files/Media/Triclosan%20-%20UKTAG.pdf (Accessed: 17/7/2017).

UKTAG (2013) *Updated Recommendations on Environmental Standards; River Basin Management (2015-21)*. [Online]. Available at: http://www.wfduk.org/sites/default/files/Media/Environmental%20standards/UKTAG%20Environmental%20Standards%20Phase%203%20Final%20Report%2004112013.pdf.

UN (2017) *World Contraceptive Use 2017*. Available at: http://www.un.org/en/development/desa/population/publications/dataset/contraception/wcu2017.shtml (Accessed: 18/02).

UNEP (2017) *Guidance for the inventory of polybrominated diphenyl ethers (PBDEs) listed under the Stockholm Convention on Persistent Organic Pollutants* Available at: http://chm.pops.int/Implementation/NIPs/Guidance/GuidancefortheinventoryofPBDEs/tabid/3171/Default.aspx (Accessed: 19/09).

UNESCO (2012) *Managing water under uncertainty and risk*.UNESCO, Paris, France. [Online]. Available at: http://www.unesco.org/new/fileadmin/MULTIMEDIA/HQ/SC/pdf/WWDR4%20Volume%201-Managing%20Water%20under%20Uncertainty%20and%20Risk.pdf (Accessed: accessed 13 Mar 2017).

Urase, T. and Kikuta, T. (2005) 'Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process', *Water research*, 39(7), pp. 1289-1300.

USEPA (1995) *Guidelines Establishing Test Procedures for the Analysis of Pollutants, 40 CFR Part 136*. [Online]. Available at: https://www.gpo.gov/fdsys/granule/CFR-2016-title40-vol25/CFR-2016-title40-vol25-part136 (Accessed: accessed 04 Nov 2015).

USEPA (2007) *Method 1614 brominated diphenyl ethers in water soil, sediment and tissue by HRGC/HRMS*. [Online]. Available at: https://www.epa.gov/sites/production/files/2015-08/documents/method_1614a_2010.pdf (Accessed: accessed 01 May 2016).

USEPA (2012) *Method 525.3: Determination of semivolatile organic chemicals in drinking water by solid phase extraction and capillary column gas chromatography/mass spectrometry (GC/MS)*. Ohio, USA. [Online]. Available at: https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=241188 (Accessed: accessed 12 Apr 2016).

USEPA (2017) *Technical Fact Sheet –Polybrominated Diphenyl Ethers (PBDEs)*. Available at: https://www.epa.gov/sites/production/files/2014-03/documents/ffrrofactsheet_contaminant_perchlorate_january2014_final_0.pdf (Accessed: 06/06).

Veetil, P.G.P., Nadaraja, A.V., Bhasi, A., Khan, S. and Bhaskaran, K. (2012) 'Degradation of triclosan under aerobic, anoxic, and anaerobic conditions', *Applied biochemistry and biotechnology*, 167(6), pp. 1603-1612.

Verlicchi, P., Al Aukidy, M. and Zambello, E. (2012) 'Occurrence of pharmaceutical compounds in urban wastewater: removal, mass load and environmental risk after a secondary treatment--a review', *Sci Total Environ*, 429, pp. 123-55.

Vieno, N., Tuhkanen, T. and Kronberg, L. (2007) 'Elimination of pharmaceuticals in sewage treatment plants in Finland', *Water research*, 41(5), pp. 1001-1012.

Vila, J., Tauler, M. and Grifoll, M. (2015) 'Bacterial PAH degradation in marine and terrestrial habitats', *Current opinion in biotechnology*, 33, pp. 95-102.

Vuono, D.C., Regnery, J., Li, D., Jones, Z.L., Holloway, R.W. and Drewes, J.r.E. (2016) 'rRNA gene expression of abundant and rare activated-sludge microorganisms and growth rate induced micropollutant removal', *Environmental science & technology*, 50(12), pp. 6299-6309.

Wang, L., Yang, Z., Niu, J. and Wang, J. (2009) 'Characterization, ecological risk assessment and source diagnostics of polycyclic aromatic hydrocarbons in water column of the Yellow River Delta, one of the most plenty biodiversity zones in the world', *Journal of hazardous materials*, 169(1), pp. 460-465.

Wang, L., Zhang, X., Tao, Y. and Hu, J. (2013a) 'Adsorption and biodegradation of typical estrogens in activated sludge under aerobic conditions'.

Wang, S., Wang, X., Poon, K., Wang, Y., Li, S., Liu, H., Lin, S. and Cai, Z. (2013b) 'Removal and reductive dechlorination of triclosan by Chlorella pyrenoidosa', *Chemosphere*, 92(11), pp. 1498-1505.

Wang, X., Xi, B., Huo, S., Sun, W., Pan, H., Zhang, J., Ren, Y. and Liu, H. (2013c) 'Characterization, treatment and releases of PBDEs and PAHs in a typical municipal sewage

treatment plant situated beside an urban river, East China', *Journal of Environmental Sciences*, 25(7), pp. 1281-1290.

Water-UK (2017) *Enrgy use and renewable energy*. Available at: https://www.water.org.uk/policy/environment/energy-use (Accessed: 24/12/2017).

Weber, S., Leuschner, P., Kämpfer, P., Dott, W. and Hollender, J. (2005) 'Degradation of estradiol and ethinyl estradiol by activated sludge and by a defined mixed culture', *Applied Microbiology and Biotechnology*, 67(1), pp. 106-112.

Weiss, F.T., Leuzinger, M., Zurbrügg, C. and Eggen, H.I.L. (2016) *Chemical Pollution in Low-and Middle-Income Countries*. Eawag.

Wenzl, T., Simon, R., Anklam, E. and Kleiner, J. (2006) 'Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union', *TrAC Trends in Analytical Chemistry*, 25(7), pp. 716-725.

WFD (2000) 'Directive 2000/60', *EC of the European Parliament and of the Council of*, 23(1).

Wu, C., Spongberg, A.L. and Witter, J.D. (2009) 'Adsorption and degradation of triclosan and triclocarban in soils and biosolids-amended soils', *Journal of agricultural and food chemistry*, 57(11), pp. 4900-4905.

Xia, X. (2013) 'Microbial degradation of polybrominated diphenyl ethers: current and future', *J Bioremed Biodeg*, 4, p. e130.

Xiang, N., Chen, L., Meng, X.-Z., Li, Y.-L., Liu, Z., Wu, B., Dai, L. and Dai, X. (2014) 'Polybrominated diphenyl ethers (PBDEs) and dechlorane plus (DP) in a conventional wastewater treatment plant (WWTP) in Shanghai: seasonal variations and potential sources', *Science of the Total Environment*, 487, pp. 342-349.

Xie, Z., Ebinghaus, R., Lohmann, R., Heemken, O., Caba, A. and Püttmann, W. (2007) 'Trace determination of the flame retardant tetrabromobisphenol A in the atmosphere by gas chromatography–mass spectrometry', *Analytica chimica acta*, 584(2), pp. 333-342.

Xue, W., Wu, C., Xiao, K., Huang, X., Zhou, H., Tsuno, H. and Tanaka, H. (2010) 'Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation', *Water Res*, 44(20), pp. 5999-6010.

Yang, Y.-Y., Borch, T., Young, R.B., Goodridge, L.D. and Davis, J.G. (2010) 'Degradation kinetics of testosterone by manure-borne bacteria: Influence of temperature, pH, glucose amendments, and dissolved oxygen', *Journal of environmental quality*, 39(4), pp. 1153-1160.

Yao, M., Li, Z., Zhang, X. and Lei, L. (2014) 'Polychlorinated biphenyls in the centralized wastewater treatment plant in a chemical industry zone: source, distribution, and removal', *Journal of Chemistry*, 2014.

Yao, M., Zhang, X.W. and Lei, L.C. (2012) 'Polycyclic aromatic hydrocarbons in the centralized wastewater treatment plant of a chemical industry zone: Removal, mass balance and source analysis', *Science China-Chemistry*, 55(3), pp. 416-425.

Yen, J.H., Liao, W.C., Chen, W.C. and Wang, Y.S. (2009) 'Interaction of polybrominated diphenyl ethers (PBDEs) with anaerobic mixed bacterial cultures isolated from river sediment', *Journal of hazardous materials*, 165(1), pp. 518-524.

Ying, G.-G. and Kookana, R.S. (2007) 'Triclosan in wastewaters and biosolids from Australian wastewater treatment plants', *Environment International*, 33(2), pp. 199-205.

Yoshimoto, T., Nagai, F., Fujimoto, J., Watanabe, K., Mizukoshi, H., Makino, T., Kimura, K., Saino, H., Sawada, H. and Omura, H. (2004) 'Degradation of estrogens by Rhodococcus zopfii and Rhodococcus equi isolates from activated sludge in wastewater treatment plants', *Applied and environmental microbiology*, 70(9), pp. 5283-5289.

Yu, C.-P., Deeb, R.A. and Chu, K.-H. (2013) 'Microbial degradation of steroidal estrogens', *Chemosphere*, 91(9), pp. 1225-1235.

Zhang, Y. and Zhou, J.L. (2005) 'Removal of estrone and 17beta-estradiol from water by adsorption', *Water Res*, 39(16), pp. 3991-4003.

Zhang, Z., Gao, P., Su, H., Zhan, P., Ren, N. and Feng, Y. (2015) 'Anaerobic biodegradation characteristics of estrone, estradiol, and 17 α-ethinylestradiol in activated sludge batch tests', *Desalination and Water Treatment*, 53(4), pp. 985-993.

Zhang, Z.L., Hibberd, A. and Zhou, J.L. (2006) 'Optimisation of derivatisation for the analysis of estrogenic compounds in water by solid-phase extraction gas chromatography-mass spectrometry', *Analytica Chimica Acta*, 577(1), pp. 52-61.

Zhao, J.-L., Ying, G.-G., Liu, Y.-S., Chen, F., Yang, J.-F. and Wang, L. (2010) 'Occurrence and risks of triclosan and triclocarban in the Pearl River system, South China: from source to the receiving environment', *Journal of Hazardous Materials*, 179(1), pp. 215-222.

Zhou, H., Huang, X., Wang, X., Zhi, X., Yang, C., Wen, X., Wang, Q., Tsuno, H. and Tanaka, H. (2010) 'Behaviour of selected endocrine-disrupting chemicals in three sewage treatment plants of Beijing, China', *Environ Monit Assess*, 161(1-4), pp. 107-21.

Chapter 8 Appendices

8.1 Appendix A: Degradation plots of the chemicals in the UK biodegradation experiment

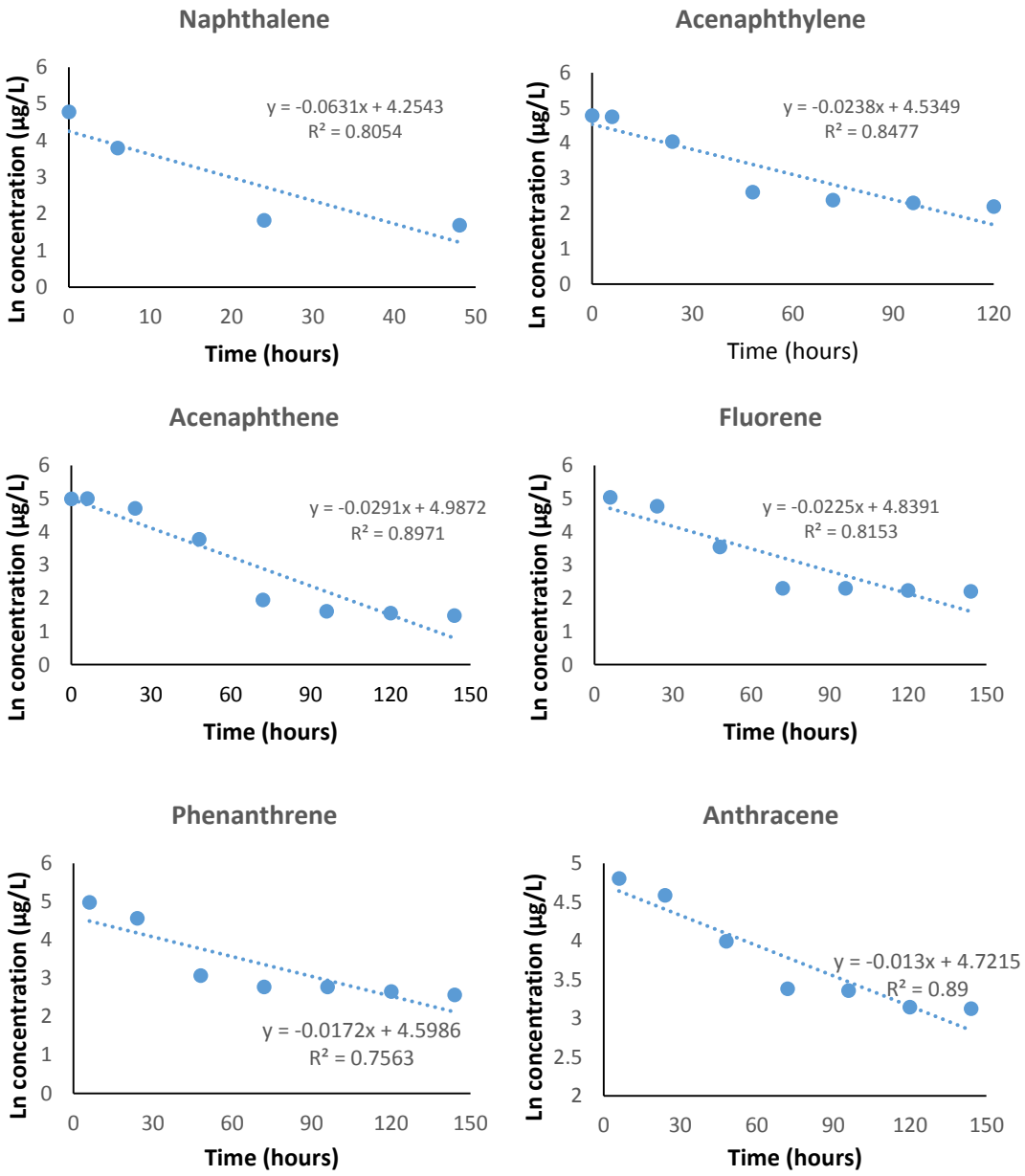


Figure 8-1 Degradation of low molecular weight PAHs over time under aerobic conditions.

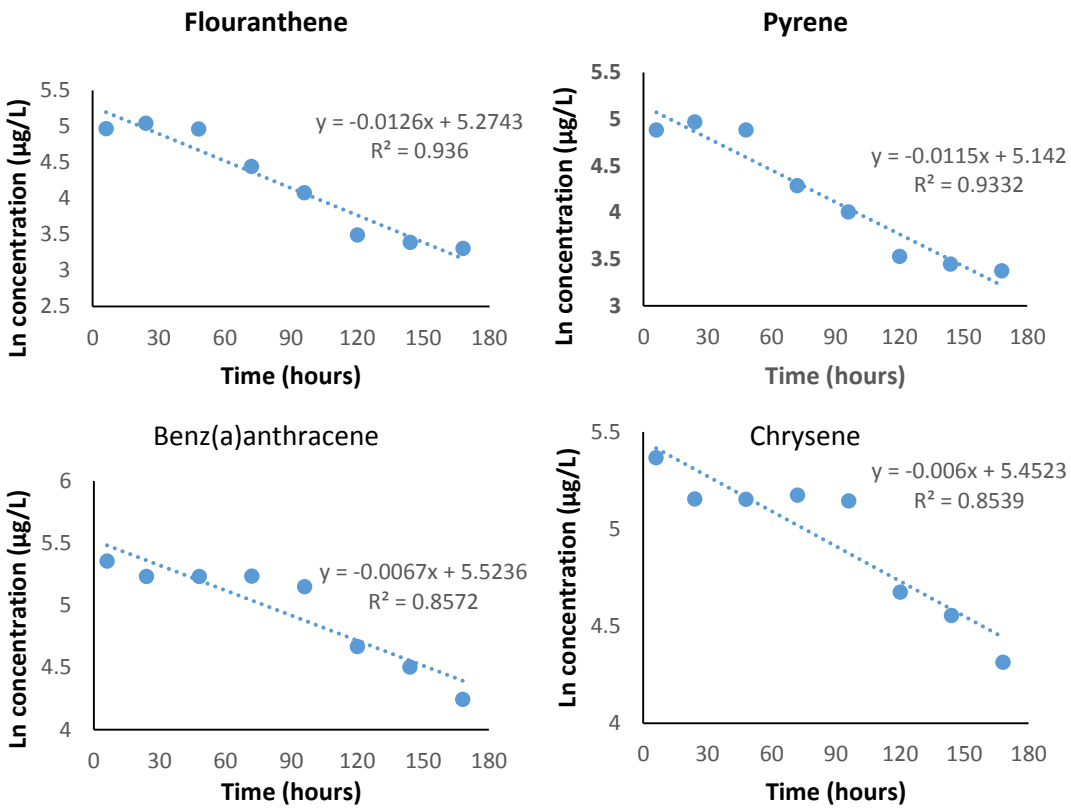


Figure 8-2 Degradation of middle molecular weight PAHs over time under aerobic conditions.

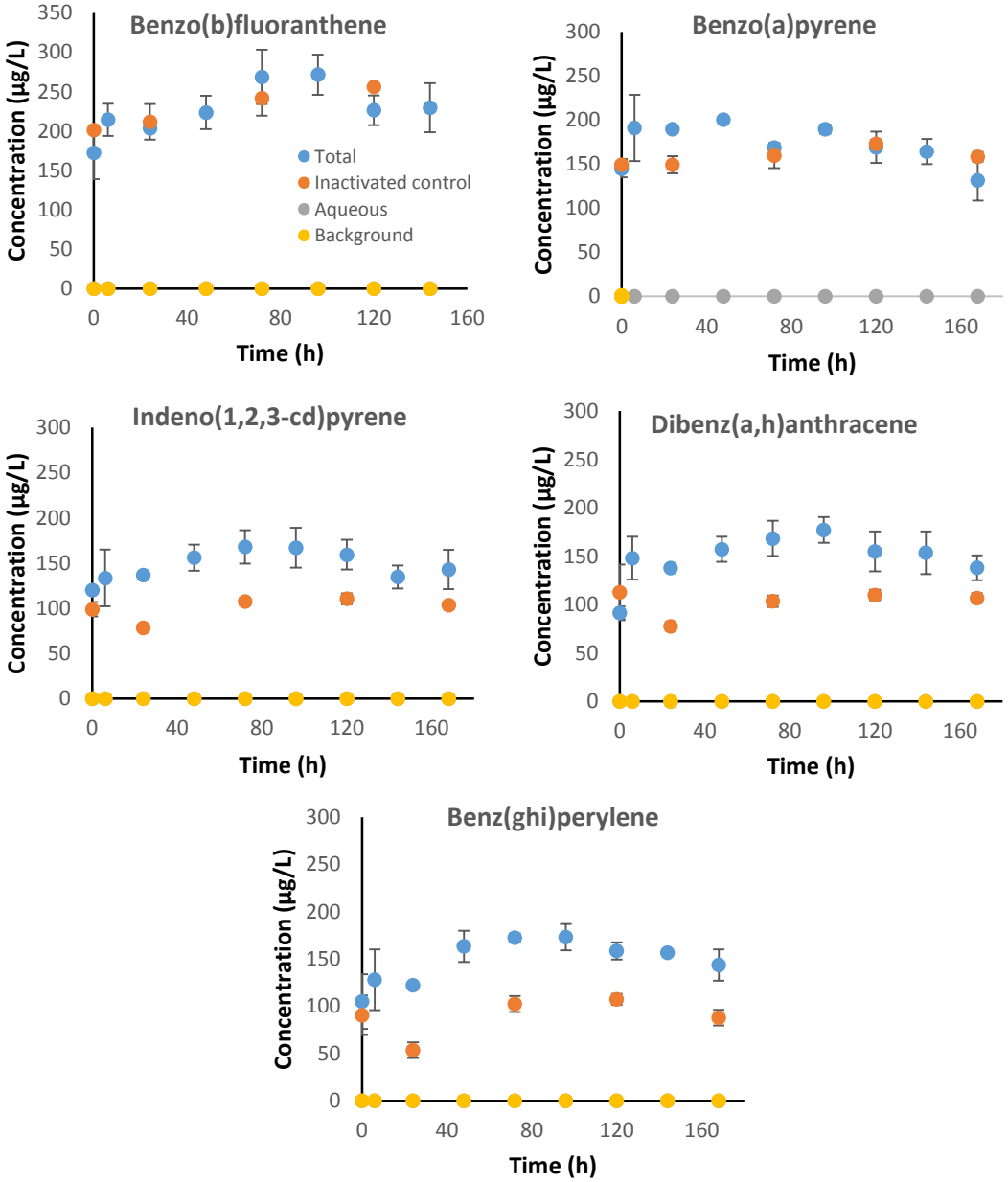


Figure 8-3 Degradation of low molecular weight PAHs over time under aerobic conditions. Error bars represents the standard deviation of triplicate measurements

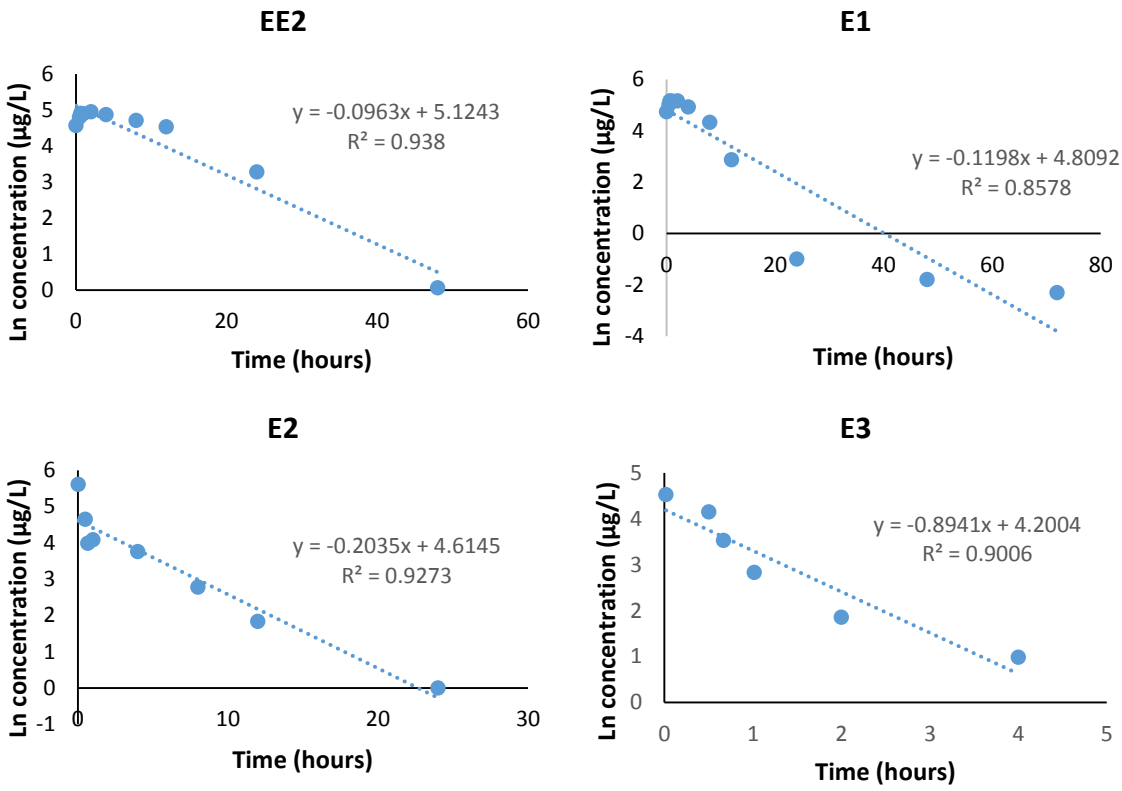


Figure 8-4 Degradation of estrogens over time under aerobic conditions.

8.2 Appendix B: Degradation charts for Brazil experiment

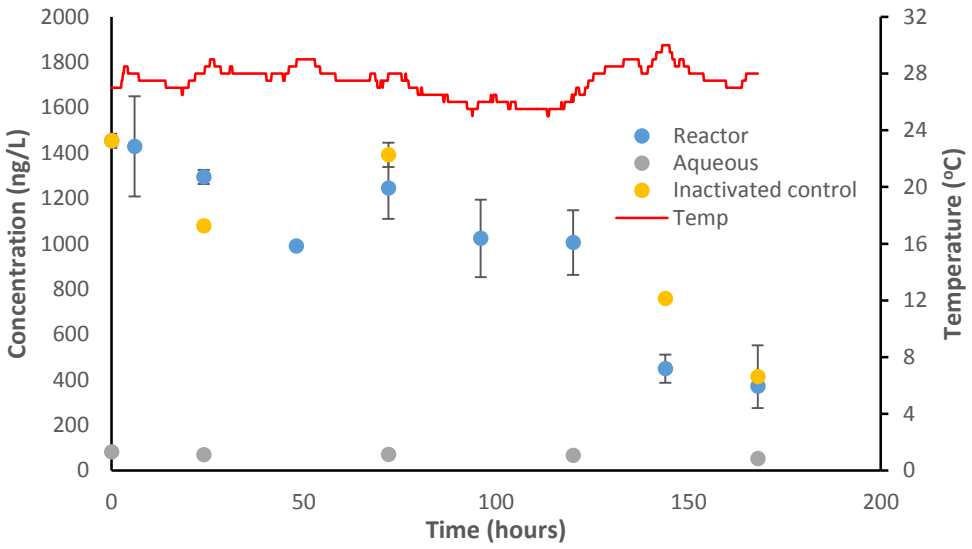


Figure 8-5 Degradation of triclosan under aerobic conditions with activated sludge inocula. Error bars represents the standard deviation of triplicate measurements

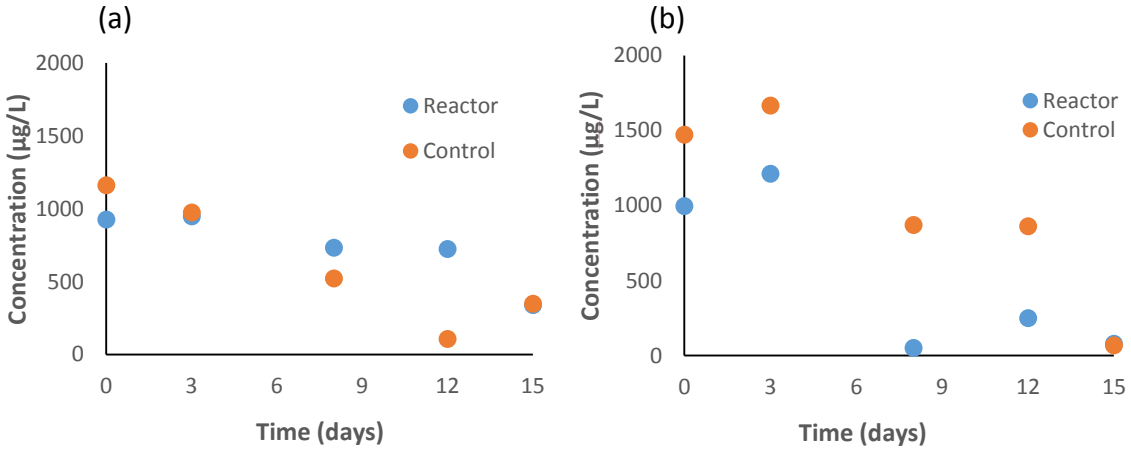


Figure 8-6 Degradation of triclosan under aerobic conditions using facultative inocula from waste stabilization pond (WSP) over time. (a) and (b) represents light and dark incubation conditions respectively

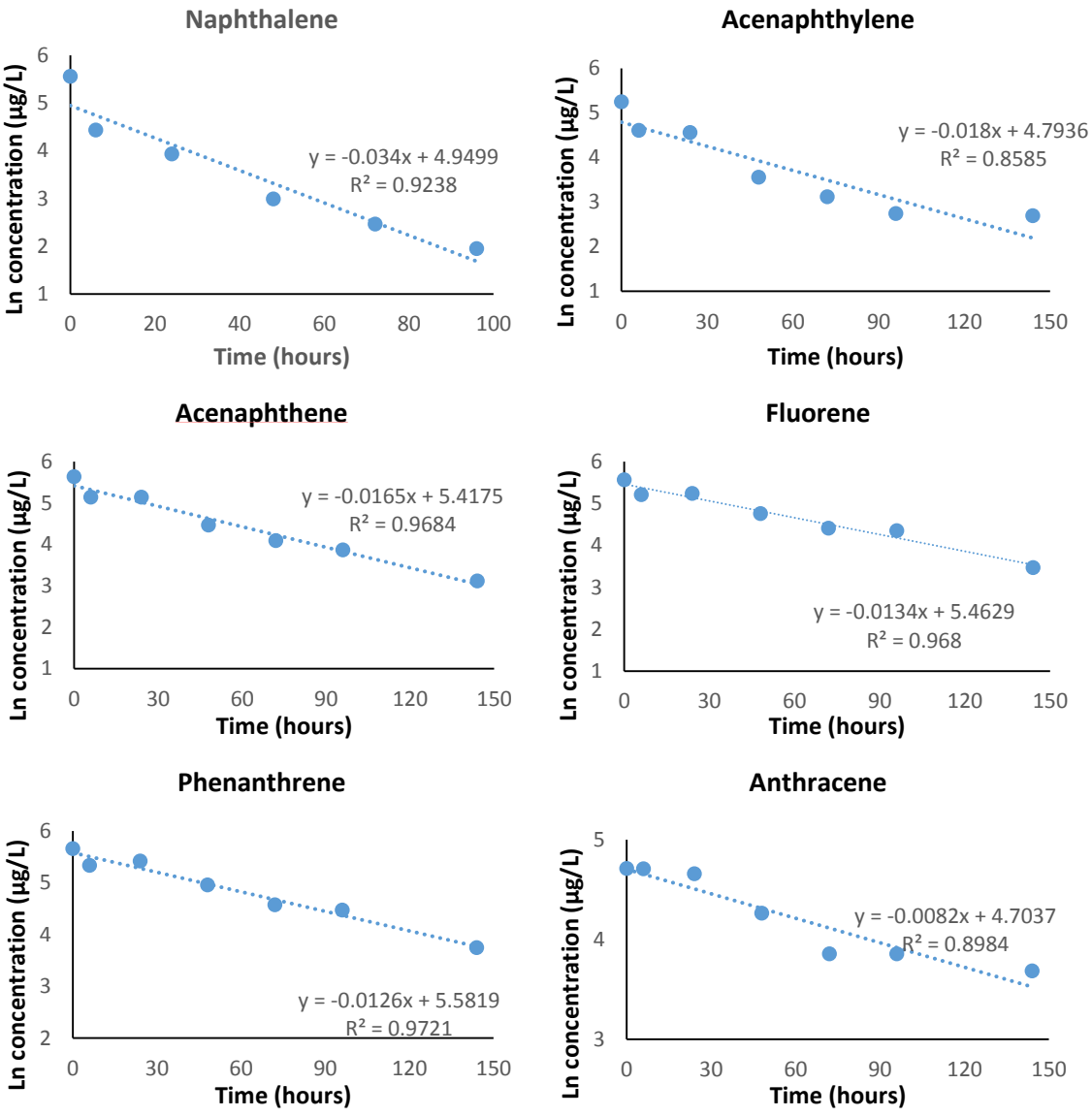


Figure 8-7 Degradation of low molecular weight PAHs under aerobic conditions with activated sludge.

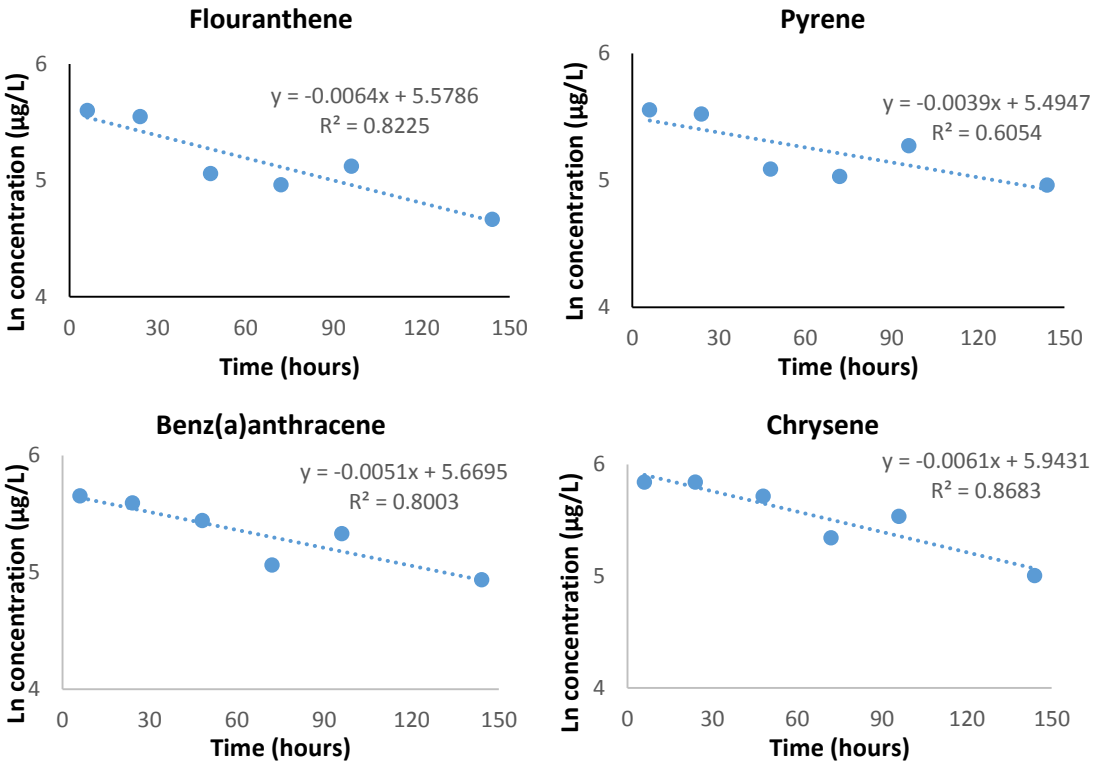


Figure 8-8 Degradation of middle molecular weight PAHs under aerobic conditions with activated sludge. Error bars represents the standard deviation of triplicate measurements

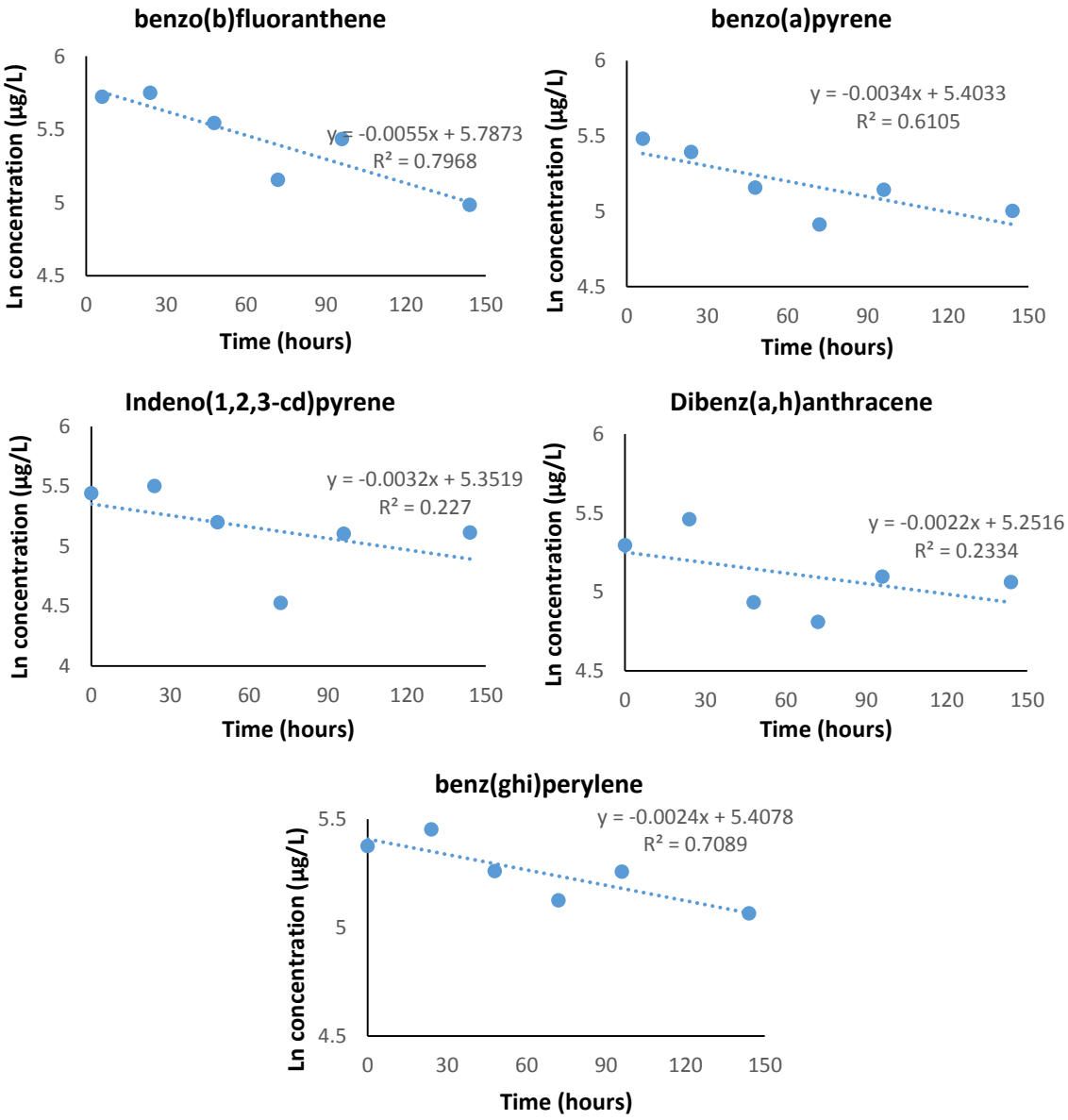


Figure 8-9 Degradation of high molecular weight PAHs under aerobic conditions with activated sludge.

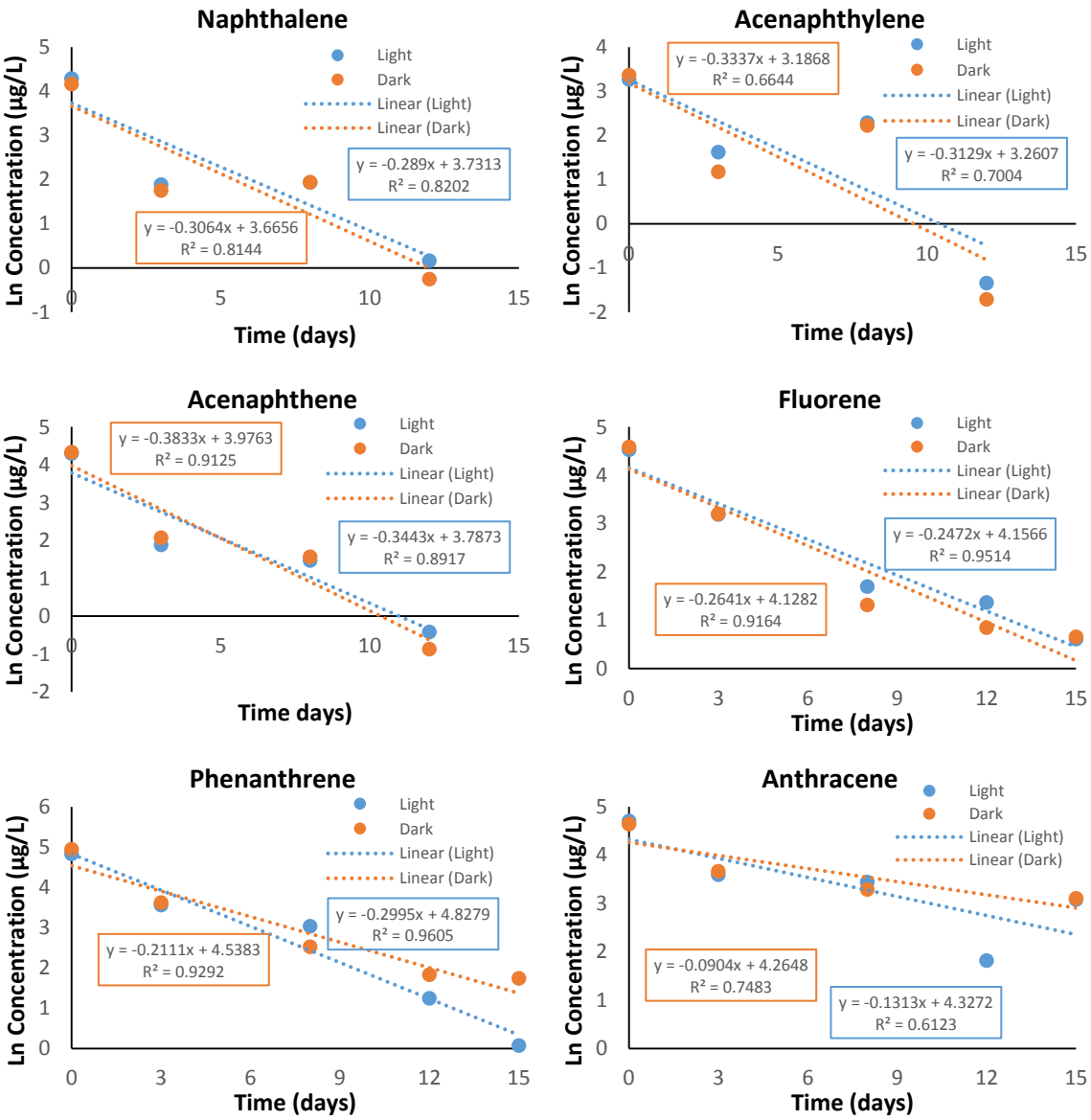


Figure 8-10 Degradation of low molecular weight PAHs under aerobic conditions with facultative pond inocula from WWTP C

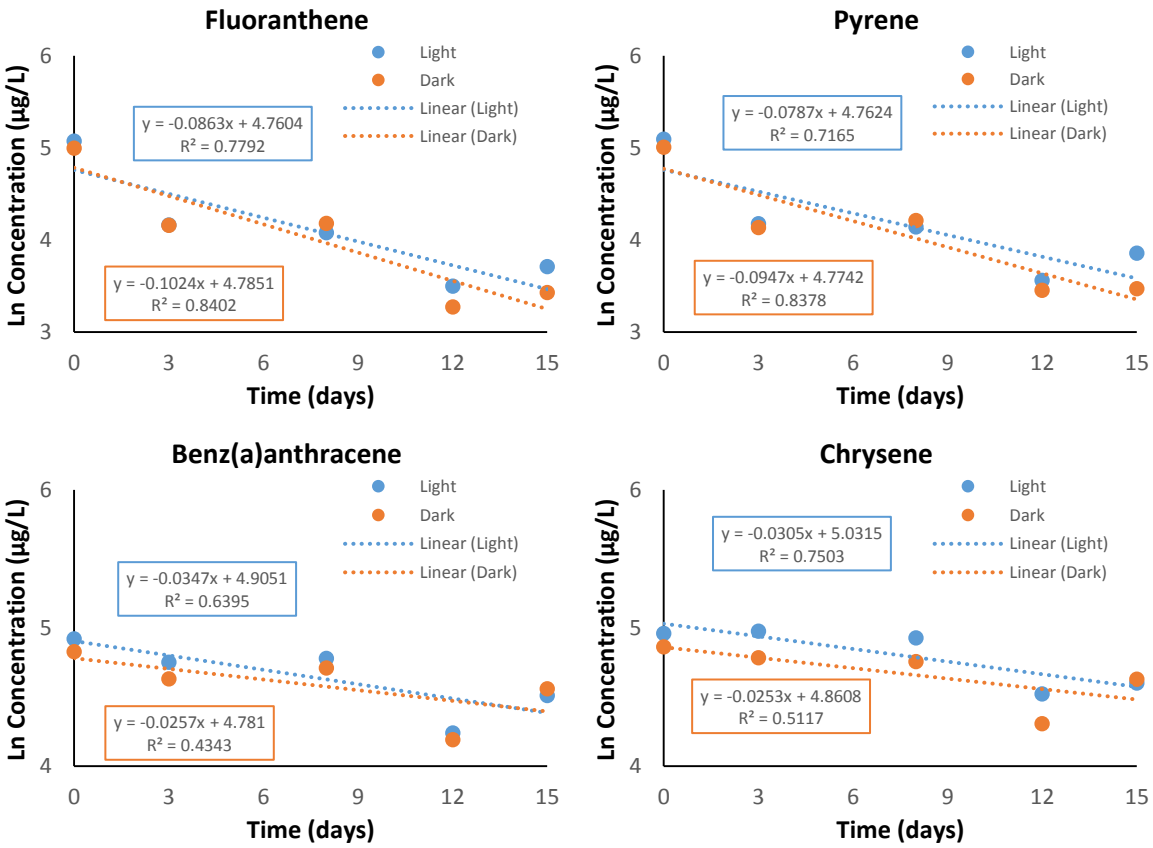


Figure 8-11 Degradation of middle molecular weight PAHs under aerobic conditions with facultative pond inocula from WWTP C

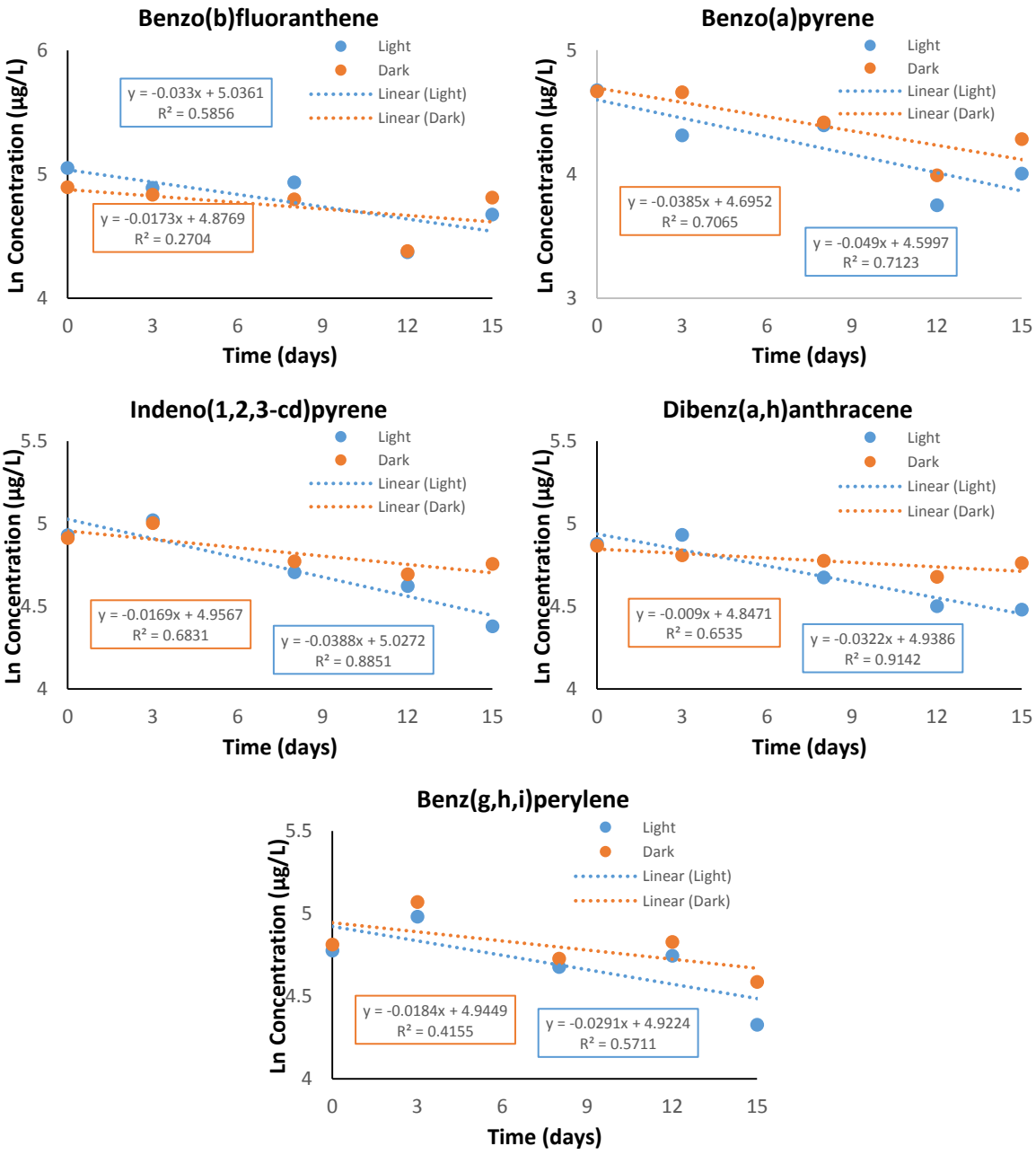


Figure 8-12 Degradation of high molecular weight PAHs under aerobic conditions with facultative pond inocula from WWTP C

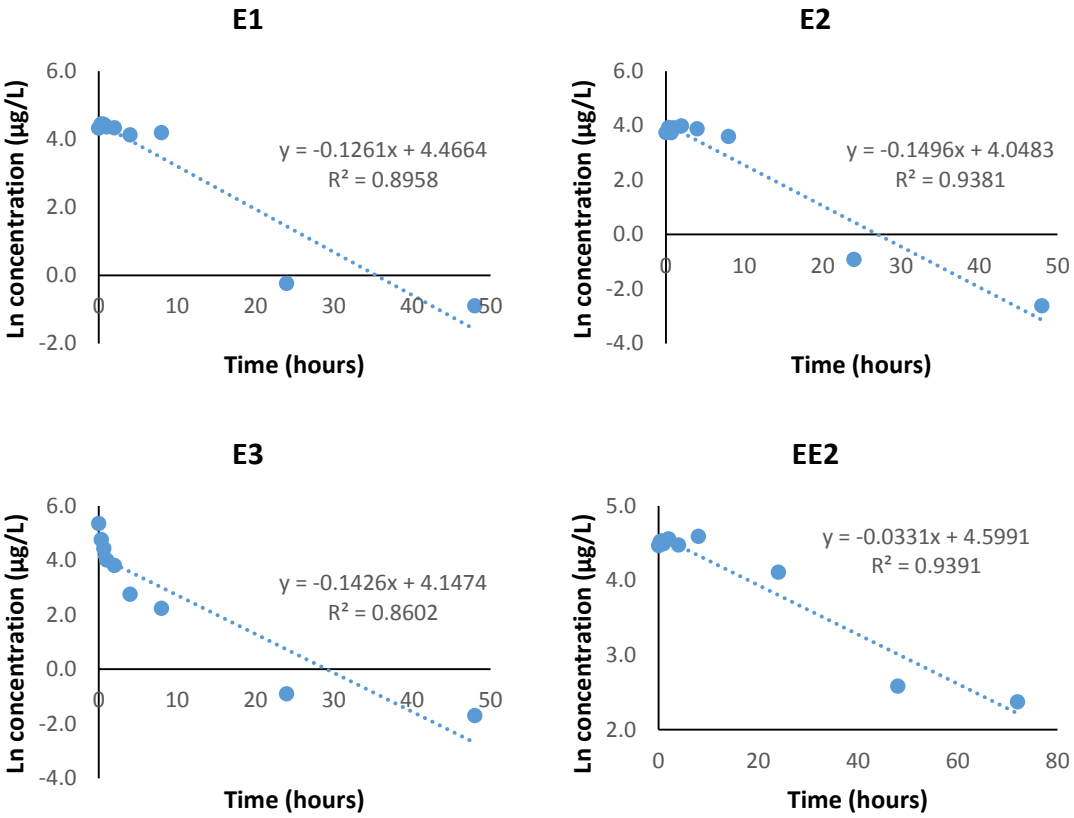


Figure 8-13 Degradation of estrogens with activated sludge under aerobic conditions. Error bars replicate standard deviation of triplicate measurements

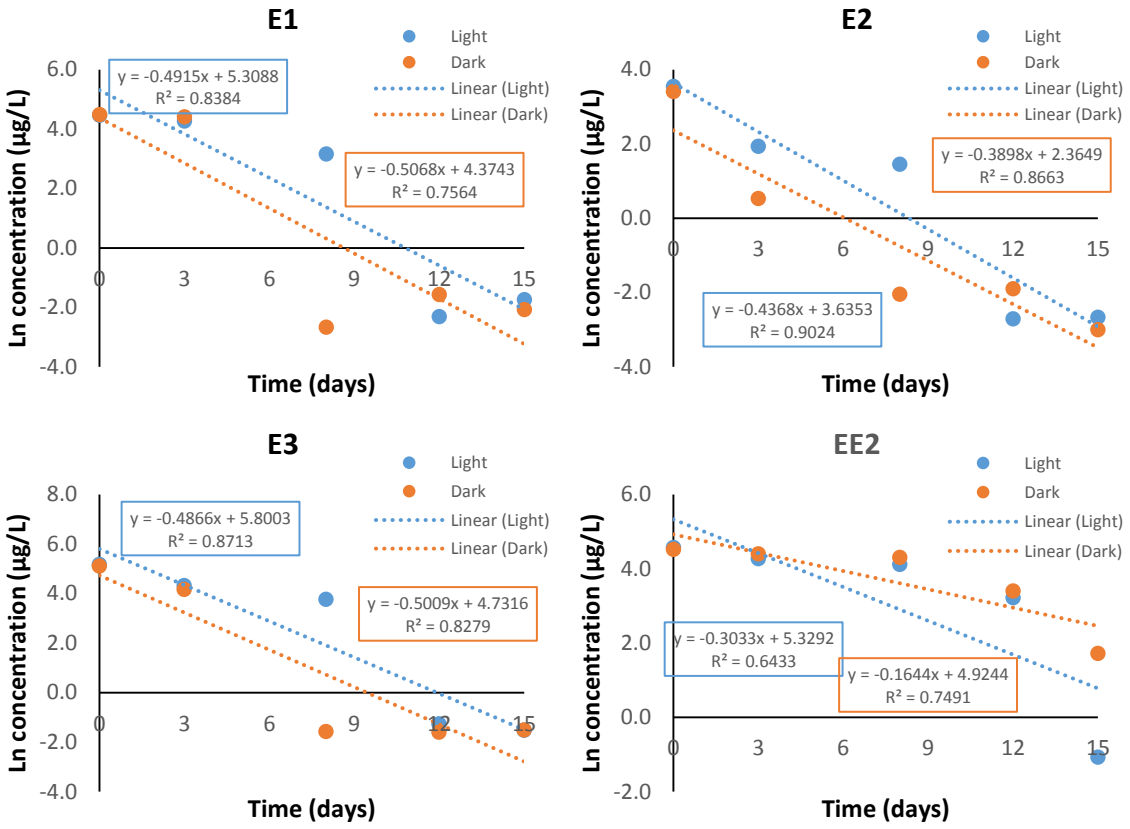


Figure 8-14 Degradation of estrogens with facultative pond inocula under aerobic conditions in the (a) Light and (b) Dark

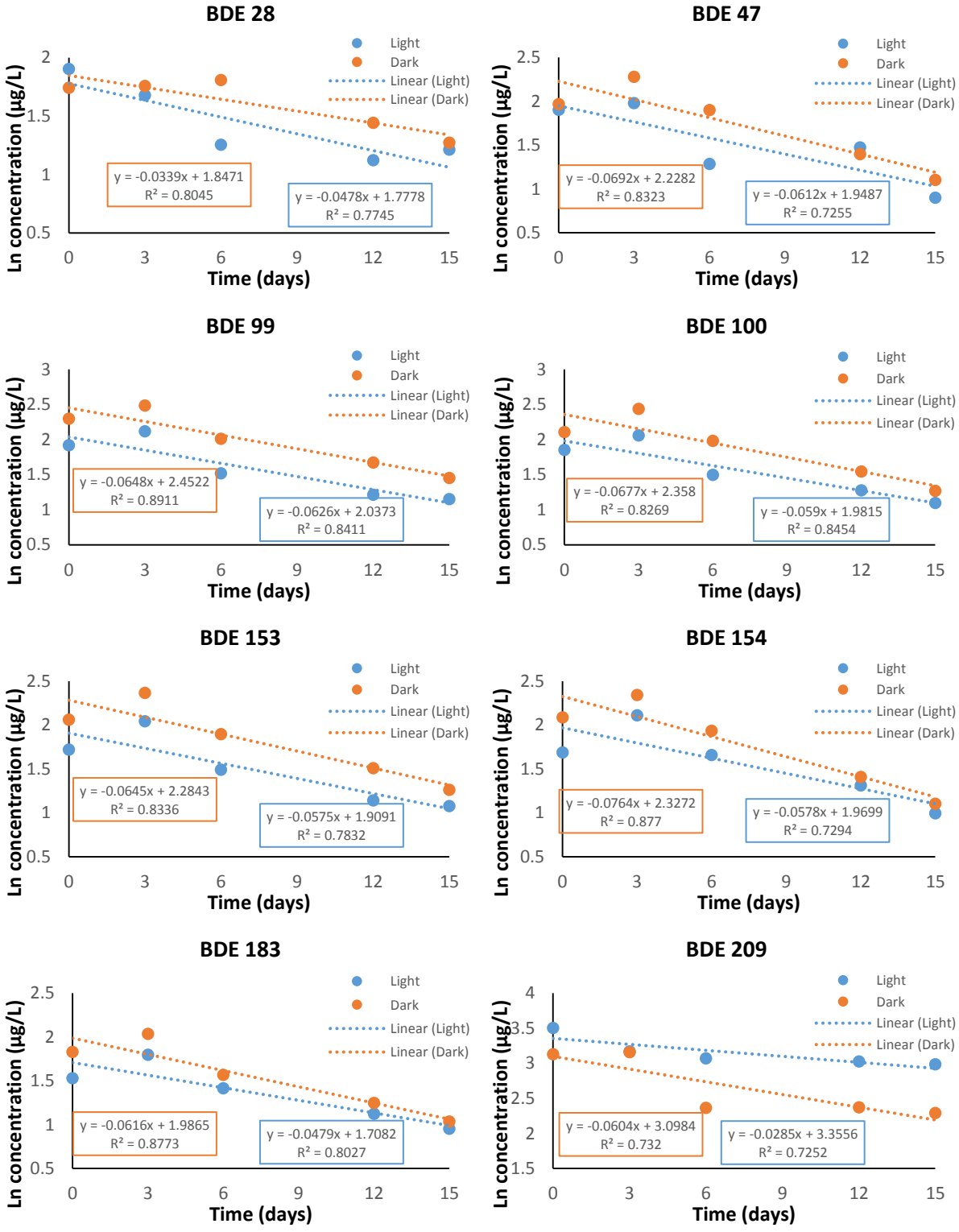


Figure 8-15 Degradation of PBDEs with facultaive inocula under aerobic conditions in the (a) light, (b) dark

8.3 Appendix C: Estimating volatilization tendency of low molecular weight PAHs

Volatilization tendency calculation

Henry's constant calculation is given as (Bamford *et al*., 1999);

$$H' = \frac{H}{RT} \qquad (1)$$

Where H = Henry's constant of a chemical at 25°C in Pa m^3/mol, H' = dimensionless Henry's constant, R = Ideal gas constant (8.314 Pa m^3/mol K) and T is absolute temperature (273 K)

$$H' = \frac{C_g}{C_w} \qquad (2)$$

C_g and C_w are gaseous and dissolved phase concentration of the chemical respectively in mol/m^3.

H for naphthalene (naph), acenaphthylene (ace), acenaphthene (acen), and fluorene (flu) is 44.6, 11.6, 16.2 and 9.8 Pa m^3/mol respectively (Shiu and Mackay, 1997).

Substituting this H values into equation (1) above, H' for naph, ace, acn and flu becomes 0.0196, 0.0056, 0.0071 and 0.0043 respectively. This equates to 1.96 %, 0.55 %, 0.71 % and 0.43 % of naph, ace, can and flu in the gaseous phase when substituted into equation (2), regardless of the dissolved concentration.

8.4 Appendix D: Volatilization rate calculation for low molecular weight PAHs

Assuming LMW PAHs volatilize via diffusion from the test vessels. Hence, according to Fick's first law, a diffusive flux will move from a region of higher concentration to a region of lower concentration across a concentration gradient

i.e. Flux = $+ A\ x\ D\ x\ \frac{C_a^{HS} - C_a^{atm}}{\Delta X}$

Where A and D = cross sectional area of the test vessel, C_a^{HS} is the concentration of the chemical in the headspace of the test vessel, C_a^{atm} is the concentration of the chemical in the atmosphere (test lab), ΔX is the distance between the headspace and atmosphere

Assuming $C_a^{atm} = 0$, A, D and ΔX are Constants

Flux = $+ Constant\ x\ C_a^{HS}$

Since $H' = \frac{C_a^{HS}}{C_w}$; where C_w is the concentration of chemical in the water, and H' is the dimensionless Henry's constant

Flux = $+ Constant\ x\ H^{'}\ x\ C_w$

The rate of disappearance of a LMW PAH is;

$\frac{dC_w}{dt} = -K_{bio}.C_w - \frac{Constant.\ H^{'}}{V_w}.C_w$. Where V_w is the volume of water

$K_{volatilization} = \frac{Constant.\ H^{'}}{V_w}$,

Hence,

$\frac{dC_w}{dt} = -(K_{bio} + K_{volatilization}).\ C_w$

$\frac{dC_w}{dt} = -K.\ C_w$ Where K = $K_{bio} + K_{volatilization}$

Hence, the first order rate equation can also be used to calculate volatilization rate. The volatilization rate were calculated from measured concentration of the chemicals in the solution phase of autoclaved control and transformed below;

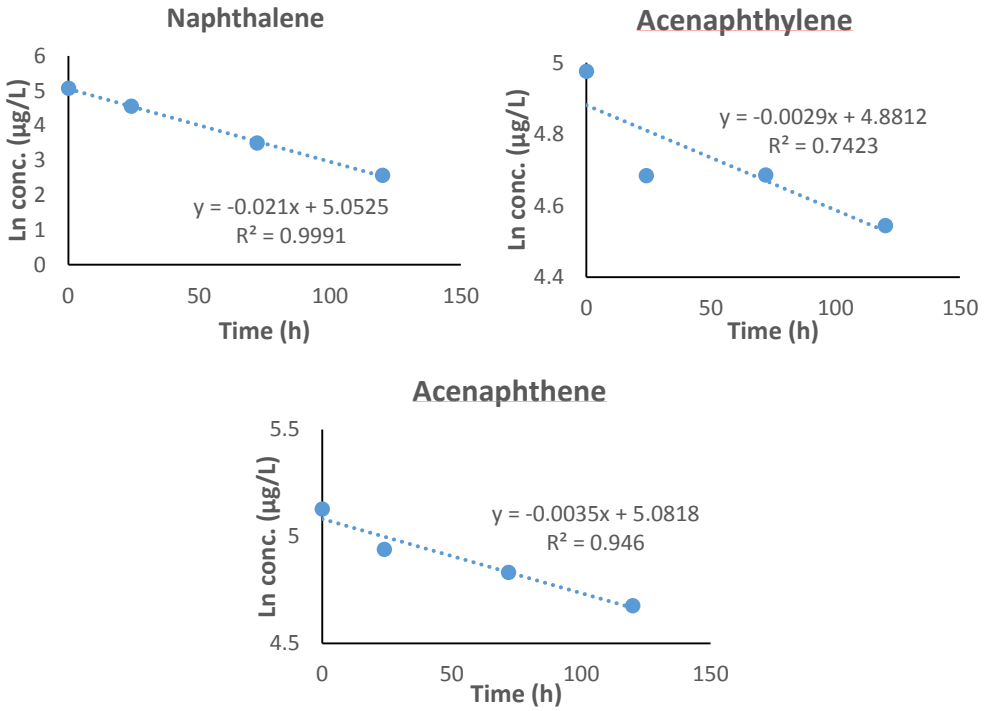


Figure 8-16 First order volatilization rate plot for LWM PAHs in the UK aerobic biodegradation experiment

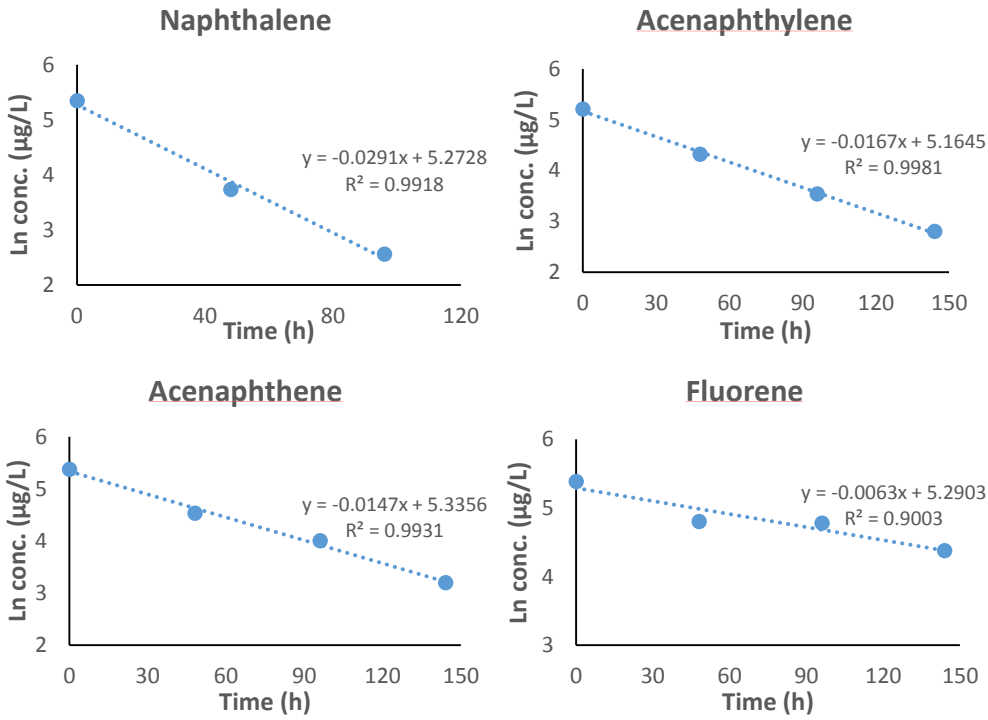


Figure 8-17 First order volatilization rate plot for LWM PAHs in the Brazil aerobic biodegradation experiment with activated sludge inocula

8.5 Appendix D: Estimating EE2 concentration in Brazil

Total population of Brazil = 206 million people (2017)

Women population in Brazil = 106 million

Reproductive women (ages 15 – 49) = 56 million (indexmundi, 2017).

% that takes all types of contraceptives = 80 %

% of those contraceptive users that takes the pill only = 34.2 % (15.3 million women, and 14.4 % of the total women population in Brazil). Hence, 7.4 % of the entire Brazil population takes the contraceptive pill.

Total load of EE2 consumed in developed countries = 27 µg/d (Johnson and Williams, 2004).

Assuming this figures are the same for Brazil,

EE2 usage per head (load) = $27\ \frac{\mu g}{d} \times\ 0.074\ = 1.92 \frac{\mu g}{d}$

Also, the amount of EE2 excreted in a study was = 10.8 µg/d (Johnson and Williams, 2004).

EE2 excreted per head (load) = $10.6\ \frac{\mu g}{d} \times\ 0.074\ = 0.78 \frac{\mu g}{d}$

To estimate the concentration of EE2 in the influent of the WWTP = $\frac{Load \times Population\ served}{Flow\ rate}$

Where load is in µg/d, flow rate in L/d.

Hence, for Pessoa et al., 2014. Population equivalent of the largest plant investigated was 23,870 and the flow was 5, 132, 000 L/d. This gives an EE2 concentration of 0.0036 µg/L (3.6 ng/L) and 8.9 ng/L for maximum excreted and usage concentration respectively.

For Froehner et al., 2010. Population equivalent of the largest plant investigated was 580, 000 and the flow was 120,900,000 L/d. This gives an EE2 concentration of 3.4 ng/L and 9.2 ng/L for maximum excreted and usage concentration respectively.

8.6 Appendix E: Estimating the theoretical oxygen demand require to mineralise the chemicals

Theoretical Oxygen Demand (ThOD)

According to OECD method 301, the theoretical oxygen of a compound ($C_cH_hCl_{cl}N_nNa_{na}O_oP_pS_s$) can be calculated by the following equation;

$$\text{ThOD} = \frac{16[2c+0.5(h-cl-3n)+3s+2.5p+0.5na-o]mg/mg}{Molecular\ weight\ (MW)}$$

For PAHs (naphthalene);

Molecular formula is $C_{10}H_8$. So every other element is 0, and MW = 128.2 g/mol

$$\text{So, ThOD} = \frac{16[2(10)+0.5(8-0-0)]mg/mg}{128.2}$$

ThoD = 3 mg of O_2/mg of Naphthalene. Since I spiked PAHs at 0.2 mg/L, ThoD becomes 0.6 mg/mg

For triclosan;

Molecular formula is $C_{12}H_7Cl_3O_2$. So every other element is 0, and MW = 289.5 g/mol

$$\text{ThOD} = \frac{16[2(12)+0.5(7-3-0)+0+0+0-2]mg/mg}{289.5}$$

ThoD – 1.3 mg of O_2/mg of triclosan (triclosan was spiked at 1 mg/L)

For estrogens (estriol-E3);

Molecular formula is $C_{18}H_{24}O_3$. So every other element is 0, and MW = 288.4 g/mol

$$\text{ThOD} = \frac{16[2(18)+0.5(24-0-0)+0+0+0-3]mg/mg}{288.4}$$

ThOD = 2.5 mg of O_2/mg of E3 or 0.25 mg of O_2/0.1mg of E3 (since 0.1 mg was spiked)

8.7 Appendix F: Calculating the Solids Retention Time (SRT) of the UASB plant in Brazil

Total wastewater flow into the plant = 147,384 m3/d

Total number of UASB reactors the flow is distributed into = 21

Hence, flow per UASB = $\frac{147{,}384}{21} = 7018.32\ m^3/d$

Total suspended solids (TSS) content in UASB effluent = 112 mg/L = 0.112 kg/m^3

Hence, total TSS load = $7018.32\frac{m^3}{d} \times\ 0.112\frac{m^3}{d} = 786.1\ kg/d$

SRT = $\frac{Sludge\ mass\ present\ in\ the\ UASB\ reactor\ (kg)}{TSS\ load\ kg/d}$

Sludge mass in reactor = 31,374.1 kg

Hence SRT = $\frac{31{,}374.1}{786.1} = 40\ days$

Typically, SRT of UASB reactors range from 30 – 50 days.

8.8 Appendix G: Removal of methyl triclosan in Brazilian WWTPs

In WWTP A, methyl triclosan was majorly removed after primary treatment (85 %), and was similar to those reported by Lozano *et al*., 2013. In WWTP B, methyl triclosan was only removed (31 %) after stage 1 secondary treatment (UASB). While the concentration of methyl triclosan decreased by 40 % after stage 1 secondary treatment (anaerobic pond) in WWTP C, it increased by 45 % after stage 2 secondary treatment (facultative pond). This result suggests that methylation of triclosan occurred in the facultative pond. However, it was interesting to observe methylation of triclosan in the facultative pond that operates under aerobic and anoxic conditions (in the upper and middle layer respectively), and not in the fully aerobic secondary treatment of WWTP A (activated sludge). Biodegradation experiments carried out using inocula from this two different secondary treatment processes to further understand this scenario. However, this might also be due to the grab samples not equating with the hydraulic load (retention times) of the systems.

8.9 Appendix H: Chromatogram of triclosan and PAHs in effluent

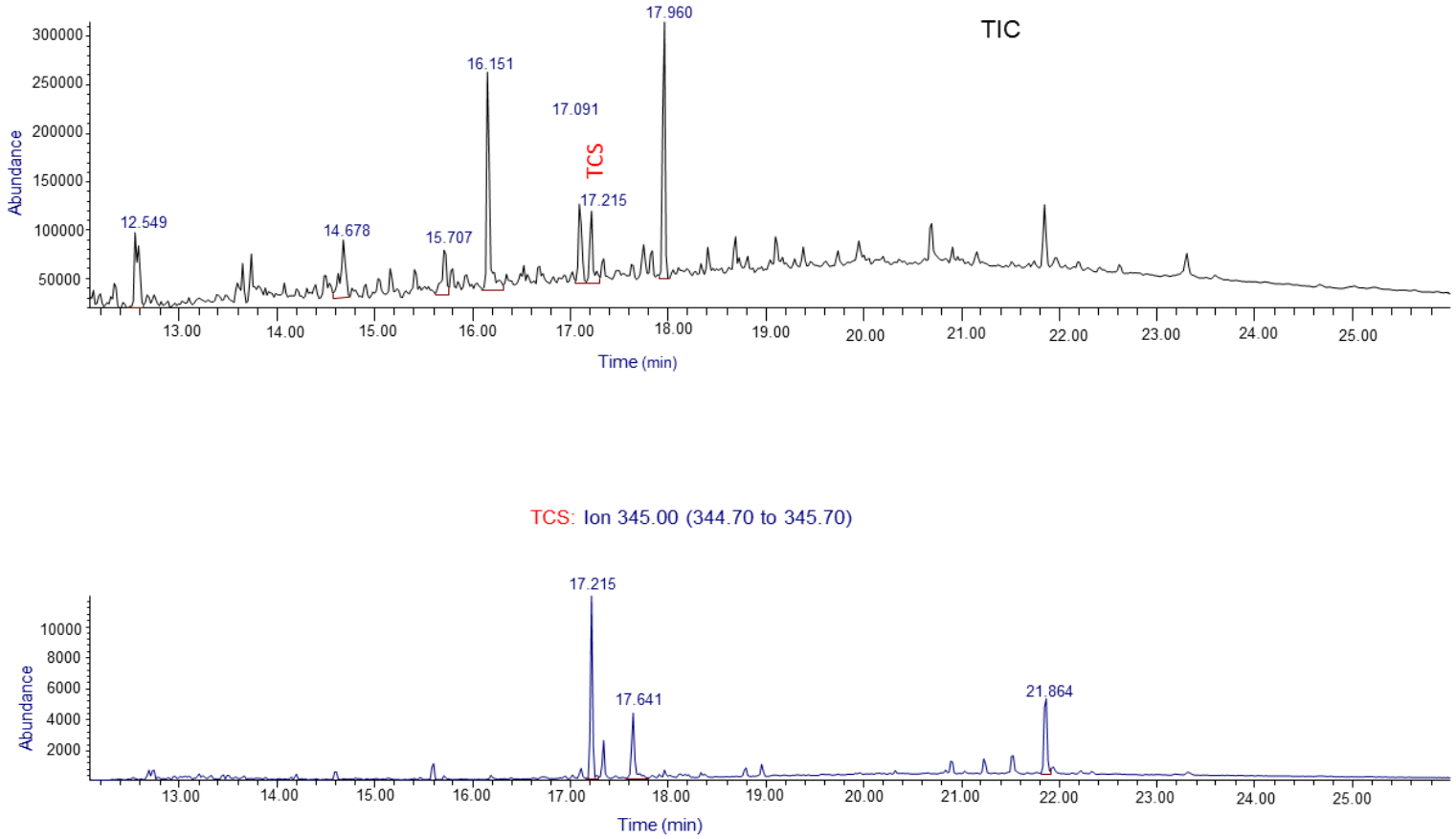


Figure 8-18 Chromatogram of effluent spiked with triclosan at 100 ng/L (above). Spectrum of the m/z ion used to quantify triclosan in the method. Triclosan was spiked before SPE

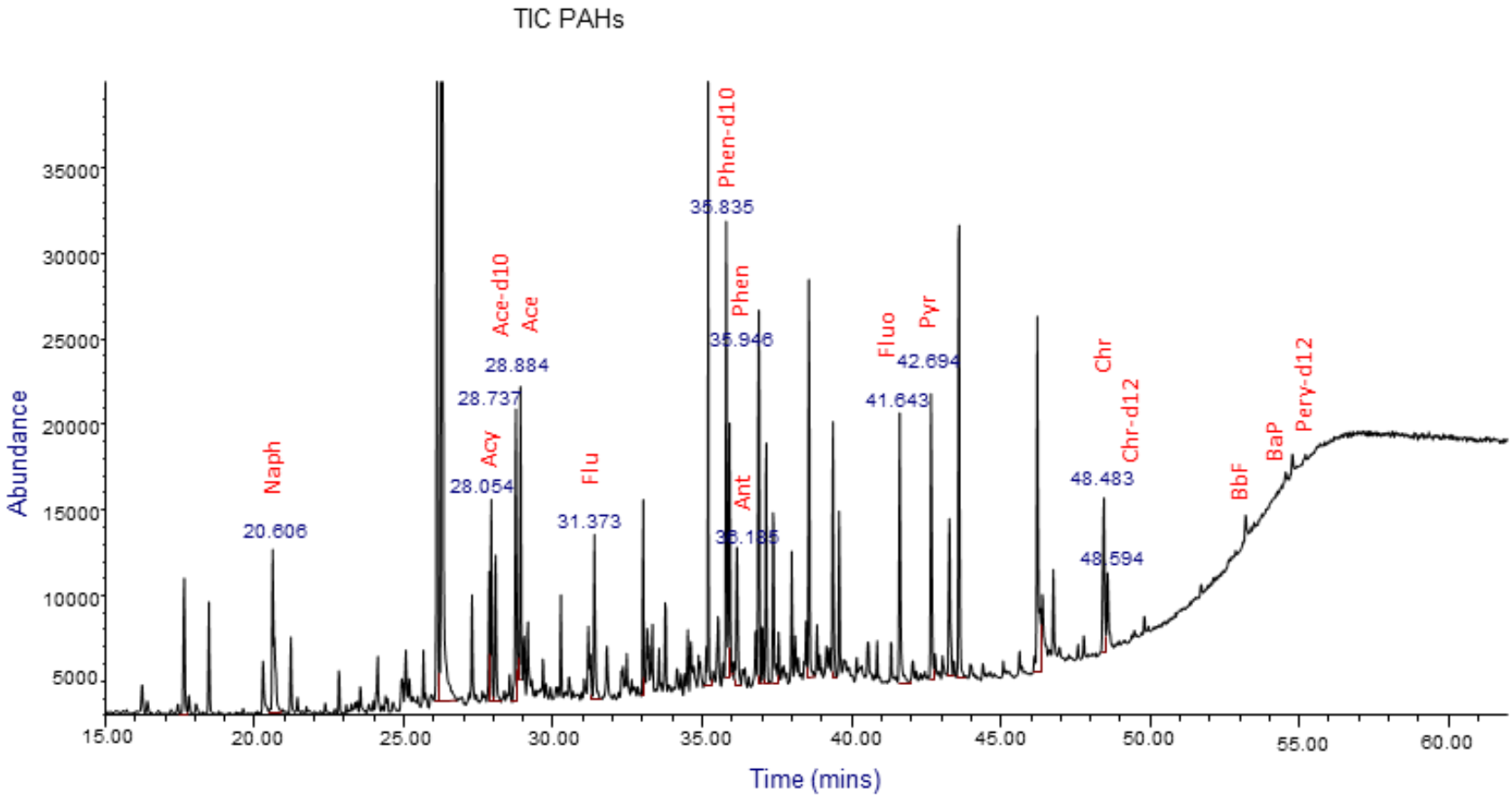


Figure 8-19 Chromatogram of effluent spiked with PAHs at 200 ng/L. The PAHs were spiked before SPE. High molecular weight PAHs were mostly visible within their m/z spectrum