Investigating the properties of persistent organic carbon in municipal wastewater treatment biosolids

A thesis submitted for the degree of Doctor of Philosophy
(PhD)

by

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Declaration

Candidate’s declarations:

I, Juliette O’Keeffe, hereby certify that this thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy (PhD), Abertay University, is wholly my own work unless otherwise referenced or acknowledged. This work has not been submitted for any other qualification at any other academic institution.

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I, Prof. Joseph Akunna, hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of Doctor of Philosophy (PhD) in Abertay University and that the candidate is qualified to submit this thesis in application for that degree.

Signed [Principal Supervisors signature]…………………………………...
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Certificate of Approval

I certify that this is a true and accurate version of the thesis approved by the examiners, and that all relevant ordinance regulations have been fulfilled.

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Abstract

Wastewater treatment plants (WWTP) are the primary pathway for many organic contaminants such as pharmaceuticals and persistent organic pollutants (POPs) found in domestic, industrial and hospital effluents to reach the environment. These substances can accumulate in WWPT sludges or treated biosolids, which are currently subject to limited environmental monitoring or regulation for organic contaminants. The lack of practical tools for assessing the relative quantities of organic contaminants and the potential for these to transfer into the environment presents a barrier to environmental regulators. The aim of this study was to improve understanding of the persistent organic composition of sludges and biosolids to better inform sludge treatment and disposal guidelines.

The study combined conventional test methods in a novel approach to assess the leachable and non-biodegradable fraction of organic carbon in sludges and biosolids to characterise how persistent organic pollutants accumulate in these matrices, and subsequently leach when applied to land. Sludges and biosolids from four municipal WWTP were investigated for desorbable dissolved organic carbon (DDOC) using leaching tests. Leachates were then assessed for their relative quantity of persistent DDOC (PDDOC) using biodegradation experiments and optical properties of both DDOC and PDDOC were examined using UV-Vis and FTIR analysis. Sludges from primary and secondary treatment stages, from both nitrifying and non-nitrifying WWTP, and processed biosolids were compared to identify the effect of treatment on relative quantities of DDOC and PDDOC. This is one of the first studies to quantify leachable and non-biodegradable DOC in municipal WWTP sludges and biosolids, comparing results between treatment plant stages and types.

The study found that there was little variability in DDOC for primary sludges but DDOC for secondary sludges varied by degree of nitrification in the WWTP, ranging from 11,760 mg.kg^{-1} in a nitrifying plant to 33,853 mg.kg^{-1} in a non-nitrifying plant. Nitrification was found to have a positive impact on reducing DDOC leached from sludges from secondary treatment stages. DDOC in biosolids undergoing thermal treatment, anaerobic digestion (AD) and dewatering were found to vary with DDOC measured at 14,422 mg.kg^{-1}, 22,542-27,862 mg.kg^{-1} and 26,155-29,983 mg.kg^{-1} respectively. Sludge treatment was found to reduce DDOC overall, with thermal treatment having the greatest effect followed by AD.

The study found that 14-39% of DDOC was found to be persistent with PDDOC values ranging from 4,096 mg.kg^{-1} in a partially-nitrifying secondary sludge to 7,547 of mg.kg^{-1} in AD treated biosolids. Concentrations of persistent mobile organic carbon at these levels warrants further consideration by environmental regulators of the potential risk associated with land application of biosolids. The levels of PDDOC were generally higher in biosolids that had undergone further treatment than in untreated primary or secondary sludges. This suggests that sludge treatment processes can result in accumulation of POPs in biosolids and may also enhance desorption potential of POPs.

The analysis of optical properties of leachates revealed a similar progression in indicators of biodegradable to non-biodegradable organic compounds between DDOC and PDDOC leachates, with an increase in the ratio of high molecular weight (MW) and aromatic compounds and indicators of functional groups consistent with some common POPs (e.g. alkyl halides, alkyl benzene compounds). The comparison of WWTP type found that non-nitrifying plants had a higher proportion of high MW, aromatic compounds than the nitrifying and partially-nitrifying plants, suggesting that extended aeration and aerobic treatment can increase removal of substances such as PAHs.

Overall the study indicates that sludges and biosolids may pose a risk of transfer of POPs into the environment through leaching of PDDOC. Determination of PDDOC could be used as a screening tool for assessing relative POP burden of sludges and biosolids and to assess the overall effectiveness of sludge treatment technologies for reducing POPs prior to land disposal. The novel approach overcomes many of the existing analytical and risk assessment barriers faced by environmental regulators providing an approach that is relatively low cost, accessible and provides a bulk measurement as an indicator of the mobile organic pollutant load.

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Definitions

Abbreviations	**Meaning**
AD	anaerobic digestion
ANOVA	analysis of variance
AOB	ammonia oxidizing bacteria
AOP	advanced oxidation processes
AOX	adsorbable organic halogen compounds
BAS	Biosolids Assurance Scheme (UK)
BDOC	biodegradable dissolved organic carbon
BOD	biological oxygen demand
BPA	bisphenol-A
BS	biosolid
CA	concentration addition
CAS	conventional activated sludge
CASS	cyclic activated sludge systems
CDC	Centre for Disease Control (US)
cm	centimetre
COD	chemical oxygen demand
CRM	certified reference material
DCM	dissolved colloidal matter
DDE	dichlorodiphenyldichloroethylene
DDOC	desorbable dissolved organic carbon
DDT	dichlorodiphenyltrichloroethane
DEHP	di-2-(ethylhexyl)phthalate
DO	dissolved oxygen
DOC	dissolved organic carbon
DOM	dissolved organic matter
dw	dry weight
E1, EE2	steroid estrogens
EPA	Environmental Protection Agency
FTIR	Fourier Transform Infrared Spectroscopy
g	gram
h	hours
HBCDs	hexabromocyclododecanes
HCB	hexachlorobenzene
HEI	highly exposed individual
HRT	hydraulic retention time
IA	independent action
K_d	desorption coefficient
kg	kilogram
KHP	potassium hydrogen phthalate
K_p	partitioning coefficient
K_{ow}	octanol:water partitioning coefficient
L	litre

Abbreviations	Meaning
LAS	linear alkyl sulphonates
LC	liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
LC-MS/MS	liquid chromatography-mass spectrometry (tandem)
LOB	limit of blank
LOD	limit of detection
LOQ	limit of quantification
M	moles of solute per litre of solution
MBR	membrane bioreactors
mg	milligram
mL	millilitre
m:m	mass:mass (ratio)
MS	mass spectrophotometry
MW	molecular weight
n/a	not applicable
n.d.	no data
NDIR	nondispersive infrared
ng	nanogram
nm	nanometre
NOEC	no observable effects concentration
NPE	nonylphenol ethoxylate
NSAID	non-steroidal anti-inflammatory drug
NSSS	National Sewage Sludge Survey
OLR	organic loading rate
OM	organic matter
OMRR	Organic Matter Recycling Regulation
OWTS	onsite wastewater treatment system
PAH	polycyclic aromatic hydrocarbons
PBDEs	polybrominated diethyl ethers
PBT	persistence bioaccumulation toxicity
PCBs	polychlorinated biphenyls
PCDDs	polychlorinated dibenzodioxins, or dioxins
PCDFs	polychlorinated dibenzofurans, or furans
PCN	naphthalenes
PCP	personal care product
PDDOC	persistent desorbable dissolved organic carbon
pe	population equivalent
PEC	predicted environmental concentration
PFAS	polyfluoroalkyl substances
PFOS	perfluorooctane sulfonate
pH	expression of the acidity or alkalinity of a solution equal to $-\log_{10}$ of the hydrogen ion concentration in moles per litre
pKa	negative logarithm of the acid dissociation constant
PNEC	predicted no effects concentration
POP	persistent organic pollutant

Abbreviations	**Meaning**
PPCPs	pharmaceuticals and personal care products
PRA	Pathway Risk Assessment
Pri	referring to primary treatment stage in a WWTP
Ref	reference material
RQ	risk quotient
SCPOP	Stockholm Convention on Persistent Organic Pollutants
SBR	sequencing batch reactors
SBLT	sequential batch leachate test
SD	standard deviation
Sec	referring to secondary treatment stage in a WWTP
SPLP	synthetic precipitation leaching procedure
SRT	solids retention time
SUVA	specific UV absorbance
SWEP	solid waste extraction procedure
TBBPA	tetrabromobisphenol-A
TCLP	toxicity characteristic leaching procedure
TF	trickling filters
TIC	total inorganic carbon
TOC	total organic carbon
UKAS	United Kingdom Accreditation Scheme
UNEP	United Nations Environment Programme
USEPA	United States Environmental Protection Agency
UV	ultraviolet
UV-Vis	spectral analysis in the ultraviolet to visible wavelength range
VOC	volatile organic compounds
vol/vol	volume to volume (ratio)
VS	volatile solids
Wt%	percent by dry weight
WWTP	wastewater treatment plant

Symbols	Meaning
°C	degrees Celsius
λ	lambda, or wavelength
μg	microgram
%	percentage
μ	mean *see Appendix 4
σ	standard deviation *see Appendix 4

CHAPTER 1 INTRODUCTION

The transfer of organic compounds into the environment from anthropogenic sources has been a cause for concern for several decades, with a diverse range of impacts on human health and the environment ensuing on both acute and chronic timescales. Globally, steps have been taken to limit the quantities of many well-known compounds such as pesticides and polychlorinated biphenyls (PCBs) from entering the environment, but large numbers of emerging compounds and new pathways of exposure mean that risks to human health and the environment are constantly evolving.

Wastewater treatment plants (WWTP) are sinks for organic pollutants from various sources including industrial effluents, hospital effluents, and domestic wastewater, all of which can contain a large and diverse collection of organic pollutants ranging from industrial chemicals and pesticide residues to emerging pollutants and pharmaceuticals and personal care products (PPCPs). WWTP are not typically designed for the removal of these pollutants, and relatively few organic compounds are regulated in municipal wastewater effluents. Growing awareness of some emerging compounds is likely to result in a greater number of compounds being regulated in the future. The Stockholm Convention on Persistent Organic Pollutants, which was the first global effort to reduce persistent organic pollutant (POP) loading into the environment, has expanded the list of candidate POPs for monitoring and regulation. The environmental pathway for many of these compounds is via wastewater treatment plants, and hence treatment plant operators will, in future, be required to monitor a greater number of pollutants in effluent and adjust treatment processes for their removal.

An unintended consequence of measures to remove additional POPs from effluent could be further concentration of these compounds in sludges. Currently, the primary removal mechanisms for organic pollutants in WWTP are sorption to sludges and biodegradation processes and municipal wastewater sludges can become an accumulation zone for many of the organic pollutants that have not been degraded. Many POPs have been measured at appreciable concentrations in municipal wastewater sludges, yet globally there is minimal environmental regulation of the organic pollutant concentrations in biosolids (McIntyre and Lester 1984; Rogers et al. 1989; Kinney et al. 2006; Smith and Riddell-Black 2007; Eljarrat et al. 2008; Diaz-Cruz et al. 2009; Edwards et al. 2010; Snyder et al. 2010; Clarke and Smith 2011; Jelic et al. 2011; Martin et al. 2012; Torri and Alberti 2012; Samaras et al. 2013; Venkatesan and Halden 2014; WCA Environment 2014; Blair et al. 2015; Semblante et al. 2015; Daughton 2016).

Municipal wastewater sludges may be disposed of via landfill or incineration, applied directly to land, or treated further and applied to land as biosolids in agriculture, horticulture or land reclamation. Land application is increasingly being considered to be an ideal disposal option amongst WWTP operators due to the economic benefits of landfill avoidance. There are also potential environmental benefits of applying biosolids to land in the recycling of valuable nutrients and organic matter. Despite the potential benefits, the land application of biosolids provides a potential pathway for pollutants such as POPs to reach the environment. Opposition to this practice is growing in some in locations such as Western Canada, where some communities perceive land application of sludges and biosolids as presenting a threat to water, soil and air quality.

Within the current regulatory framework for sludges and biosolids in most countries, trace metals and pathogens form the primary focus of monitoring, regulation and additional treatment. Much less consideration is given to the presence of organic pollutants, with the reasons for this linked to the absence of practical tools for risk assessment and monitoring of organic pollutant transfer into the environment. Barriers to development of these tools include:

- analytical challenges preventing cost-effective and timely analysis of POPs in sludge and biosolid materials;
- practical difficulties in monitoring the vast number of possible organic pollutants that could be present;
- the lack of practical tools for assessing the relative pollutant burden of complex mixtures;

- the lack of research on the effect of treatment parameters on reducing organic pollutant concentrations in sludges and biosolids;
- the lack of appropriate indicators of risk.

Bulk quantification measures for POPs are not currently used in environmental monitoring, with current practice focussed on individual compounds or groups of similar compounds (e.g. PCBs). Quantifying or setting environmental limits for total POP or indicators of POP load is currently not carried out. There has also been limited study of the migration of POPs from biosolids into the environment, although leaching studies are often carried out to determine possible migration of other types of pollutants such as trace metals compounds into the environment, as indicators of environmental risk.

With no regulatory driver for WWTP to reduce POP loads in sludges, there is little incentive to investigate or adapt processes to enhance POP removal amongst wastewater treatment operators. However, as legislation begins to be enacted for emerging compounds in effluents, the pollutant burden in sludges and biosolids may increase as effluent removal processes enhance partitioning to sludges. This could also increase the environmental risks associated with land application of sludges and biosolids. More research is needed to understand the partitioning of POPs into municipal wastewater treatment sludges and to better understand the impact of treatment processes on organic pollutant burden. Given the barriers listed above, it is currently difficult for operators to predict how the changes in operational controls or treatment technologies will impact the POP burden in sludge and biosolids or to monitor changes over time due to lack of practical indicators or measurements of effectiveness.

Suitable monitoring and assessment tools are needed to enable treatment plant operators to improve understanding of the effect of wastewater treatment processes on reducing POP loads in sludges and biosolids and to inform regulators about the potential risks of land application of sludges and biosolids. This study seeks to expand the knowledge on persistent organic compounds in municipal wastewater sludges and biosolids by investigating the migration of dissolved organic carbon from these materials and evaluating the relative persistence of the desorbable, dissolved organic carbon (PDDOC). The study will assess whether various wastewater treatment processes impact the quantity of persistent organic carbon that is partitioned to sludges during wastewater treatment processes and assess whether a non-specific measure (PDDOC) could be used as an indicator of organic pollutant migration from sludges and biosolids applied to land.

1.1 Research Aims and Objectives

1.1.1 Aim

The overall aim of the work is to investigate the desorbability and persistence of dissolved organic carbon (DOC) in sludges and biosolids from various types of municipal wastewater treatment plants (WWTP).

1.1.2 Objectives

The objectives of the study are as follows:

- To develop a replicable protocol for the determination of persistent desorbable dissolved organic carbon (PDDOC) in WWTP sludges or biosolids;
- To determine and compare the level of desorption of organic carbon from sludges and biosolids;
- To determine and compare the relative biodegradability of organic carbon desorbed from sludges and biosolids;
- To establish relationships between persistent desorbed organic carbon and presence of persistent organic pollutants;
- To inform practical environmental management guidelines for the assessment, treatment and handling of sludges and biosolids to reduce risks to human health and the environment from persistent organic pollutants.

1.2 Structure of the Thesis

The thesis will be presented as follows: Chapter 1 provides the background and introduction to the study. Chapters 2 to 5 present a review of the literature and an evaluation of the state of research and knowledge of persistent organic contaminants in WWTP sludges and biosolids. The literature review is structured as follows:

Chapter 2 presents a review of the literature relating to POPs in the environment. This is followed by an overview of the occurrence of POPs in WWTP and the impact of wastewater treatment processes on POP reduction. A discussion of the likely impacts of various operational parameters and treatment stages on removal of organic pollutants is presented. Chapter 3 presents a review of the literature concerning the composition of sludges and biosolids and current disposal practices and regulation. The occurrence and fate of POPs in sludges and biosolids is examined, with a consideration of the evidence of organic pollutant transfer into the environment from these materials. The influence of DOC on pollutant transfer is considered. The approaches to environmental risk assessment of organic pollutants is presented with a consideration of how these methods could apply to POPs in sludges and biosolids. Chapter 4 presents an examination of the literature relating to alternative approaches for quantifying environmental risks associated with land application of sludges and biosolids. This includes a review of approaches to assessment of leaching potential, quantitative techniques for measurement of POPs, and qualitative analysis techniques. Chapter 5 synthesises the key findings of the literature review.

Chapter 6 presents the experimental design used in the research. This includes a description of the approach to the experimental work, site selection, sample collection, preparation and processing. This section also presents the general analytical approaches used, with detailed methods presented in relevant chapters that follow. Chapters 7 to 9 present the results from the three analytical components of the study. Chapter 7 presents the results of the study to determine the desorbable DOC (DDOC) from sludges and biosolids obtained from various types of municipal WWTP. Chapter 8 presents the results of the biodegradation experiments to determine persistent desorbable dissolved organic carbon (PDDOC). Chapter 9 presents the qualitative examination of optical properties of sludges and biosolids using UV-Vis and FTIR to examine the DDOC and PDDOC profiles of selected sludge and biosolid samples. Chapter 10 present a discussion of the implications of the results to management of environmental risks associated with land application of sludges and biosolids. Chapter 11 provides the key conclusions of the work and identifies recommendations for future study.

CHAPTER 2 LITERATURE REVIEW: PERSISTENT ORGANIC POLLUTANTS AND WASTEWATER TREATMENT PROCESSES

2.1 Introduction

This chapter presents a review of the literature relating to persistent organic pollutants (POPs) in the environment. This is followed by an overview of the occurrence of POPs in WWTP and the impact of wastewater treatment processes on POP reduction. A discussion of the likely impacts of various operational parameters and treatment stages on removal of organic pollutants is presented.

2.1.1 A history of organic pollutants and environmental regulation

Organic chemicals have been fundamental to the advancement of modern civilisation. Chemists have been experimenting with alterations to the basic organic form of carbon-based compounds for well over a century and have created substances capable of treating human illness, enhancing agricultural production, and providing new products and methods of manufacturing. The economic and social benefits of new organic substances resulted in widespread increase in their use and continued development of new chemicals. As early as 1943, the production of organic chemicals in the United States was already approximately 4.5 million tonnes, increasing to 64 million tonnes in 1972 with 12000 commercial chemicals in use in the United States in 1974 and up to 20,000 in the United Kingdom in 1982 (Bedding *et al.* 1982). The increase in production, use and disposal, led to the environment becoming a sink for many substances only developed within the last century. In the early 1960s, some of the environmental and human health impacts of organic pollutants in the environment were documented in the well-known book “Silent Spring”, written by Carson (1962). She wrote:

> “*For the first time in the history of the world, every human being is now subjected to contact with dangerous chemicals, from the moment of conception until death*”.

Carson documented many accounts of the environmental and human health effects resulting from organic pollutants, particularly chlorinated hydrocarbon pesticides such as dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE). Both acute and chronic effects on wildlife populations and humans were reported by Carson, along with description of persistence in the environment and the bioaccumulation, biomagnification and biotransformation processes that can amplify the potential harm from exposure. Although Carson received widespread criticism for her alarmist writings about compounds such as DDT, her book proved important to raising awareness of the potential impacts of unabated release of organic chemicals into the environment, which had previously not been considered in many cases.

Since the 1960s, there has been increasing recognition of the potential unintended consequences of unabated release of organic chemicals into the environment. Organochlorine pesticides (e.g. DDT) were some of the first POPs to be banned starting in the 1970s (Muir and Howard 2006). Organisations such as the US Environmental Protection Agency (EPA), the Food and Drug Administration, and the National Cancer Institute in the US began to consider screening of organic compounds as possible carcinogens, mutagens, or causes of other health effects to establish regulatory guidelines for their release into the environment (Stephenson 1977). The Toxic Substances and Control Act (TSCA) 1976 was a product of this process. Other countries also introduced legislation limiting the release of chemical substances, such as the Chemical Substances Control Law in Japan (1973), the European EC-Existing Substances legislation (1981), and the Domestic Substances List in Canada (1986). In the late 1970s and 1980s, restrictions on use took effect for substances such as polychlorinated biphenyls (PCBs), widely used in industry between the 1960s and 1980s in electrical components, pump fluids, additives, diluents and flame retardants (Bracewell *et al.* 1993; Muir and Howard 2006). Restrictions were accompanied by environmental monitoring and measuring techniques for some compounds as analytical capabilities improved. Progress was seen in the form of sharp decreases in concentrations of PCBs in municipal wastewater sludges and bird eggs in the early 1990s (Chaney *et al.* 1996; Braune *et al.* 2019).

Since early detection of organic contaminants in the environment, more sophisticated and refined analytical methods and detection equipment have enabled a wider range of compounds, across multiple matrices, to be identified and quantified at lower levels of detection than previously possible. In addition, application of ecotoxicological testing has provided tools for the scientific community to quantify the potential for harm from environmentally relevant concentrations of pollutants. Despite this progress, today there are relatively few organic compounds that are regulated in the environment in comparison to the increasing number and diversity being released into the air, water and soils. Progress on reducing environmental concentrations of regulated compounds slowed towards the end of the 1990s and into the 2000s (Braune *et al.* 2019). As concentrations of compounds regulated in the initial phases of regulation reduced due to removal from use, there was less urgency behind regulation of some emerging organic pollutants. Many unidentified organic compounds however were being detected in environment samples such as river waters (Hendriks *et al.*, 1994) with an increasing diversity of emerging pharmaceuticals and personal care products, illicit drugs, drug metabolites and transformation by-products entering the environment via wastewater treatment effluents (Baker *et al.* 2012).

In 2001, recognising the potential impact on human health and the environment from persistent organic pollutants, the United Nations Environment Programme (UNEP) established the Stockholm Convention on Persistent Organic Pollutants (SCPOP) to encourage signatories to prohibit, eliminate or restrict use and production of listed POPs, and implement measures to increase awareness, carry out monitoring and research and establish non-compliance measures (UNEP 2015). The SCPOP entered into force 17 May 2004, and as of 2017, 181 countries were signatory (Fiedler *et al.* 2013; UNEP 2017). It is now widely recognised that the properties of organic compounds that are potentially most harmful to the environment include persistence in the environment, ability to bioaccumulate, and ability to cause toxic effects (El-Shahawi *et al.* 2010; Venkatesan and Halden 2014; Mansour *et al.* 2016). These characteristics (persistence, bioaccumulation and toxicity, or PBT) were used to identify candidate pollutants for inclusion in the SCPOP. The primary aim of the SCPOP is to eliminate dangerous POPs, starting with the twelve worst (the “dirty dozen”), which include DDT, PCBs and a series of pesticides and industrial chemicals, most of which were discussed by Carson in the early 1960s. Slow progress has been made to add new compounds to the SCPOP, and the process of identifying, assessing, and agreeing regulatory approaches for individual compounds is extremely slow and inefficient. In 2017 sixteen new compounds were added to the SCPOP (UNEP 2017). These include pesticides, industrial chemicals and unintentional by-products.

Compared to the vast number of possible organic pollutants, the number regulated under the SCPOP is a relatively small. UNEP estimate that up to 140,000 chemicals are now traded on the European market (UNEP 2013) and the chemical abstracts services inventory contains almost one billion compounds and continues to grow (Anumol *et al.* 2015). The EU NORMAN network has now identified over 700 emerging pollutants in Europe's aquatic environment (Geissen *et al.* 2015) and emerging organic compounds are also now being detected in human blood, serum, urine and tissues in developed countries (CDC 2009). As use of some compounds is phased out, such as those identified in the initial SCPOP, it is likely that environmental concentrations will reduce, however as thousands of potential emerging pollutants are released into the environment the consequences for ecosystem and human health remain relatively poorly understood (Onesios *et al.* 2009). Obstacles to quantifying the risk to human health and the environment from these emerging compounds include the expanding list of new compounds being discharged into the environment, poor understanding of the environmental significance of compound mixtures and the potential risk from metabolites or transformation products of parent compounds (Chaney *et al.* 1996). Study of the potential environmental and human health risks from exposure to emerging compounds in the environment is still relatively scarce for some compounds. Effects can range from acute or chronic toxicity, enzyme inhibition leading to physiological effects, reproductive effects or even behaviour change, which can have population level and evolutionary impacts (Küster and Adler 2014). Pollutants such as pharmaceuticals have been observed to bioaccumulate in lower trophic levels such as benthic invertebrates in wastewater-impacted river systems, providing potential for transfer up food chains, with possible ecosystem-level effects (Grabicova *et al*. 2015).

For pharmaceutical substances such as hormones and antimicrobials, and compounds used in manufacturing such as polybrominated and polyfluorinated compounds or by-products of industrial processes such as dioxins and furans, the body of evidence regarding possible risks of harm is growing. Table 2-1 summarises some of the known risks associated with exposures to organic pollutants in the environment, which may occur via exposure in freshwater or soil environments for flora and fauna or in drinking water or contaminated food for humans.

Table 2-1. Review of environmental and human health risks of selected organic compounds including pharmaceuticals and industrial additives and by-products.

Compound type	Risks to the environment	Risks to human health	Reference
Selected pharmaceuticals			
diclofenac (non-steroidal anti-inflammatory drug (NSAID))	renal disease and visceral gout amongst bird populations feeding on affected food sources		Oaks *et al*. (2004)
ibuprofen (NSAID)	adverse metabolic effects on aquatic and terrestrial organisms		Samaras *et al*. (2013)
cyclophosphamide (anti-cancer drug))	toxicity in fish including liver and kidney impairment, DNA damage; effects on plant viability and propagation	genotoxic, mutagenic and reproductive toxicity at relatively low concentrations	Heath *et al*. (2016); BIO Intelligence Service (2013)
carbamazepine (anti-epileptic drug)		evidence of carcinogenicity after chronic exposure in rats	Schricks *et al*. (2010)
metformin (diabetes medication)	persistent endocrine disrupting compound, decreased plant growth and causes intersex in fish after chronic exposure		Briones and Sarmah (2019)
iomeprol (contrast media)		adverse effects on liver and kidney	Schricks *et al*. (2010)
hormones: e.g. estrogens, ethinylestradiol (EE2), 17β-estradiol	effects on hormonal system in non-target organisms such as fish, molluscs, invertebrates; feminization of fish, impaired reproduction, intersex causing population level impacts	endocrine disruption and possible diverse effects on reproduction and sexual development such as declining sperm counts, reproductive disorders related with male infertility, testicular cancer, and breast cancer	Küster and Adler (2014); BIO Intelligence Service (2013); Houtman (2010); Mompelat *et al*. (2009)
antimicrobials: e.g. antibiotics, anti-virals, disinfectants, parasiticides	toxic effects on non-target organisms such as aquatic invertebrates, insects, and protozoa affecting ecosystem stability	antimicrobial resistance in human gut flora	BIO Intelligence Service (2013); Küster and Adler (2014); Larsson (2014)
lindane (insecticide/headlice treatment)	immuno-toxic, reproductive and developmental effects on animals and aquatic organisms	potential neuroendocrine and developmental toxicity	UNEP (2017)

Compound type	Risks to the environment	Risks to human health	Reference
Manufacturing additives and industrial by-products			
hexabromocyclododecane (HBCDs)		potential neuroendocrine and developmental toxicity	UNEP (2017)
polybrominated diphenyl ethers (PBDE)	toxicity to mammals	toxicity to humans, disruption of thyroid functioning, endocrine disruption	UNEP (2017); Houtman (2010)
polyfluoroalkyl substances (PFAS)		toxicity to humans; developmental effects, possible carcinogens probable links with high cholesterol, thyroid disease, pregnancy-induced hypertension, ulcerative colitis, kidney and testicular cancer; possible effects on immunity, and renal function in children	Houtman (2010) Sunderland *et al*. (2019)
bisphenol A (BPA)		reproductive toxicity, endocrine disruption, carcinogenic effects, cardiovascular disease, type 2 diabetes, liver enzyme abnormality.	Mohapatra *et al*. (2010); Houtman (2010)
PCBs	toxicity to fish, reproductive failure and immune system suppression in mammals	neurodevelopmental effects, probable human carcinogen; possible links to type-2 diabetes	UNEP (2017) Wu *et al*. (2013) Everett *et al*. (2010)
naphthalene		dermal effects in humans, liver disease following chronic exposure, suspected cancer risks.	UNEP (2017)
dioxins (PCDDs) and furans (PCDFs)	toxic effects in laboratory animals including reproductive impacts	adverse effects on human immune system, enzyme disorders, possible human carcinogens.	UNEP (2017)
1,4-dioxane		possible carcinogen	Schricks *et al*. (2010)

Table 2-1 continued

2.1.2 Wastewater treatment plants: the pollutant super-highway

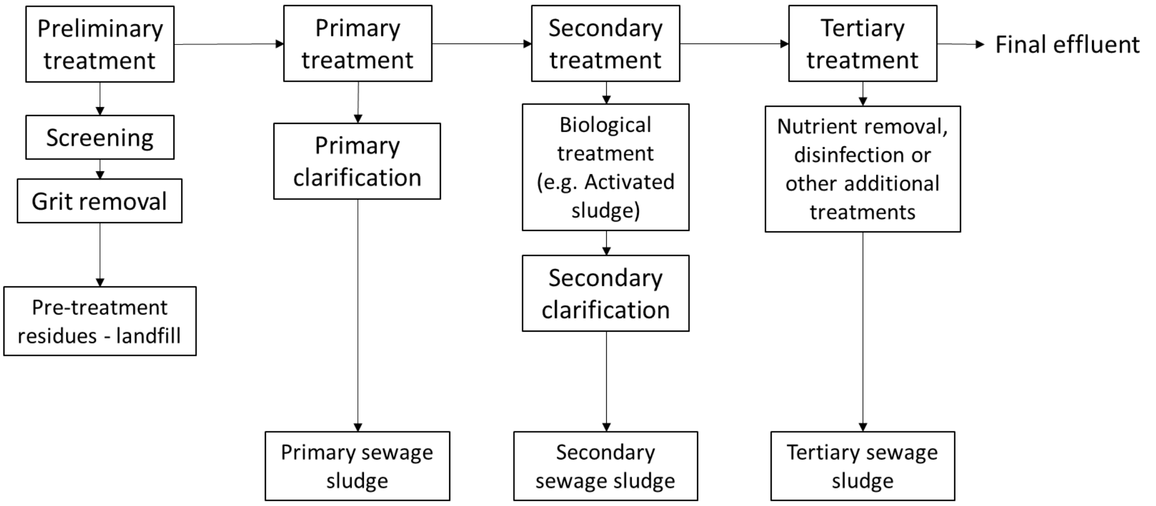
Wastewater treatment plants (WWTP) receive effluents from multiple sources including household, industrial and hospital effluents and road and surface run-off. Many emerging organic compounds such as those found in household and pharmaceuticals and personal care products (PPCPs) are now being observed in wastewater treatment effluent and detected widely in the environment with potential impacts on ecosystems and human health (Monteith *et al.* 2010; Wu *et al.* 2013; Geissen *et al.* 2015). WWTP are recognised as the major pathway for many PPCPs to reach the environment (Liu and Wong 2013). da Silva *et al.* (2011) found that in a study of six WWTP in Spain, and associated upstream and downstream concentrations in rivers, the concentrations of pharmaceutical compounds were highest downstream of WWTP, indicating the contribution of WWTP to environmental concentrations of POPs. Similarly, Baker and Kasprzyk-Hordern (2013) found that analysis of river water upstream and downstream of WWTP in England revealed the presence of 29 of the 64 compounds monitored (mostly illicit drugs) with an increased cumulative concentration of all monitored compounds downstream of WWTP. Some compounds such as pharmaceuticals reach the environmental only via WWTP, and it can now be demonstrated that these compounds are persisting in the environment and making their way into either food or water supplies. For example, a study of drinking water by the Associated Press in 2008 found that more than 41 million US citizens were exposed to one or more active pharmaceutical ingredients in their drinking water, with these pharmaceuticals reaching the environment almost exclusively via WWTP (Halden 2010).

A major challenge for environmental regulation of POPs for the protection of human health and the environment is the pace at which new and alternative compounds are being developed. For example, polybrominated diphenyl ethers (PBDEs), a POP recently added to the SCPOP, is known to be persistent, toxic and bioaccumulative, and therefore is being phased out of use as a flame retardant in materials like textiles and carpets (Hwang *et al.* 2012; Zeng *et al.* 2014). However, the need for flame retardant treatments in industry is still present, and other chemicals have begun to take the place of PBDEs, such as hexabromocyclododecanes (HBCDs) and tetrabromobisphenol-A (TBBPA). These compounds are less well studied but are suspected endocrine disruptors with possible neurotoxic effects (Hwang *et al.* 2012). HBCDs have now also been added to the SCPOP but are still permitted for use in some applications. Polyfluoroalkyl substances (PFAS), used in a wide range of industrial applications from photo processing to fire-fighting foams, are now widely detected the environment, ground water and in blood and tissues of humans and wildlife (Sunderland *et al.* 2019). Their potentially toxic effects (Table 2-1) have resulted in a selection of these chemicals being regulated in drinking water in Canada and some US states, however new replacements (e.g. the Gen-X chemicals) are being developed to replace them in industrial processes, with unknown effects on human health or the environment. Development of replacement chemicals to overcome regulatory barriers can lead to a cycle of regulation chasing chemical development, with a lag phase between use and regulation. The development of new analytical methods does not always keep pace with the emergence of new compounds, and the complexity of the sludge matrix and compound mixtures present challenges to regulators to put regulatory limits on individual compounds (Paterakis *et al.* 2012). Given the potential for WWTP to be major sources of the next generation of emerging pollutants, greater understanding of the effect of wastewater treatment processes on environmental loading of emerging pollutants is needed. The next section considers the key principles of current wastewater treatment approaches and how various processes and operational parameters impact the fate of organic pollutants.

2.2 Wastewater treatment processes and organic pollutants

2.2.1 Principles of wastewater treatment

Current municipal WWTP are typically designed to provide, at a minimum, removal of solids and biodegradable organic carbon from wastewater effluents. Configuration of treatment plants can vary significantly depending on several factors such as the receiving environment, the regulatory regime, the size of the population and types of wastewater sources entering the plant, as well as the economic and human resource capacity of the community to implement complex or advanced treatments. Most treatment plants include an initial screening process to remove large contaminants and grit removal to remove large heavy particulates through sedimentation. Some treatment plants then provide only basic primary treatment, designed to achieve clarification by settlement of solids or air floatation and removal of surface scum. Following clarification, effluent may be discharged to the environment in many cases or sent for secondary biological treatment in a range of configurations to remove biodegradable compounds. Following secondary treatment, a second stage of clarification may be undertaken, which can be enhanced by chemical addition (e.g. alum, ferric chloride or polyacrylamide) to promote settlement and in some cases followed by tertiary treatment where additional nutrient removal or disinfection is carried out. Figure 2-1 depicts a typical treatment plant configuration. At each treatment stage, there is removal of solids. These solids may be eliminated separately but are often combined for further processing. This can include dewatering plus treatments to reduce pathogens and odour (heat treatment or lime addition) but typically there is no specific treatment provided to remove chemical contaminants.



Adapted from NRC 2002

Figure 2-1 Wastewater treatment plant processes (Adapted from NRC 2002)

Aside from the configuration of the process steps, plant operators may alter operational parameters such as hydraulic retention time (HRT, or the time required for influent to become effluent), or solids retention time (SRT, or sludge age) depending on the throughput and capacity of the system and requirement for additional treatment. Increasingly WWTP carry out ammonia removal to meet regulatory conditions for their municipal wastewater discharge into the environment. In order to achieve this, WWTP may incorporate processes such as aeration to encourage nitrification (microbiological conversion of ammonia/ammonium to nitrite, and then nitrate) followed by additional processes (e.g. an anoxic tank) to encourage denitrification (microbiological conversion of nitrate to nitrogen gas (N_2)). Nitrification requires suitable conditions for nitrifying bacteria (e.g. nitrosomas, nitrobacter) that include adequate O_2 concentrations, and ideal pH (e.g. slightly alkaline) and the rate of nitrification increases with temperature (EPA 1974). Denitrification requires anoxic conditions such that facultative denitrifying bacteria utilise nitrate as an oxygen source, and thus facilitate the conversion of nitrate to N_2.

2.2.2 Organic pollutant removal in wastewater treatment

The study of organic pollutants in wastewater treatment processes did not gain much attention until the 1980s (Rogers 1996). Within a treatment system, organic compound removal can be categorised as mass fluxes to air due to volatilisation, mass fluxes to water (effluent) and mass fluxes to sludges (Nyholm *et al.* 1996). These mass fluxes are created by various processes including volatilisation, adsorption to solids, biodegradation/transformation and abiotic degradation/chemical degradation (Rogers 1996; Lin *et al.* 2010, Delgadillo-Mirquez *et al.* 2011; Geissen *et al.* 2015; Barbosa *et al.* 2016). These processes may not be independent, with abiotic processes such as sorption-desorption limiting the bioavailability and hence the rate of biodegradation or conversely the production of enzymes by microorganisms influencing chemical transformation processes. Various wastewater treatment processes will affect the degradation and removal of organic compounds as well as partitioning between different compartments of the wastewater matrices such as influent, effluent, particulate and sludge phases (Geissen *et al.* 2015; Petrie *et al.* 2015). A description of the main removal processes of sorption, biodegradation and abiotic removal are presented below followed by a discussion of the processes that dominate each stage of wastewater treatment.

2.2.2.1 Sorption

Sorption to solids is an important removal process for organic compounds in wastewater treatment systems. While partitioning to solids can prevent some organic compounds from being removed by volatilisation, chemical or microbial degradation during the treatment process, for highly persistent compounds sorption may be the only mechanism preventing these compounds from reaching the environment in effluent (Wang and Jones 1994; Jelic *et al.* 2011). Many POPs have been shown to concentrate in wastewater treatment sludges. Jelic *et al.* (2011) assessed 43 target compounds in WWTP sludges and found 21 compounds to be found at significant concentrations of the magnitude of 100 $mg \cdot kg^{-1}$. In a survey of biosolids from WWTP across the US, Venkatesan and Halden (2014) detected 123 organic pollutants of the 231 that were assessed including brominated flame retardants, antibiotics, surfactants, amongst others. The literature suggests that different types of sludges, generated under different operating conditions may contain varying levels of persistent organic carbon and there are a number of factors that affect the degree of sorption and subsequent desorption from solids (Katsoyiannis and Samara 2005, Haynes 2009).

2.2.2.1.1 Hydrophobicity

Early understanding of partitioning of organic contaminants in municipal wastewater focussed particularly on the octanol-water partition coefficient (e.g. K_{ow}), or the degree of hydrophobicity, as the most important characteristic of a compound to determine it's partitioning from the aqueous phase of sludge effluent to the solid phase. Rogers (1996) suggested that chemicals with log K_{ow} less than 2.5 (e.g. hydrophilic) have low sorption potential; between 2.5 and 4 have medium sorption potential and greater than 4 (e.g. hydrophobic) have high sorption potential. Byrns (2001) describes the rate of adsorption of hydrophobic non-polar organics as a linear function with the K_{ow}. Morissette *et al.* (2015) observed that pharmaceutical compounds with log K_{ow} >3 showed sorption to sludges in the first 5 minutes of sorption experiments and low sorption was observed in the first 6 h for low log K_{ow} compounds (e.g. <3) such as caffeine, sulfamethoxazole, desethylatrazine, and carbamazepine. This confirms Rogers (1996) and Byrns (2001) suggestion that hydrophobic substances with a high log K_{ow} compounds will sorb more readily to sludges compared to hydrophilic compounds. This rule, however, is very general, and can only partially explain sorption processes. For example, Morissette *et al.* (2015) showed that while sorption of hydrophilic substances did not occur in the initial stages of processing, substantial sorption of some such compounds (e.g. sulfamethoxazole) was observed after 6 h.

2.2.2.1.2 Other factors affecting partitioning

Sorption to solids can be affected by more than just hydrophobic partitioning (McPhedran *et al.* 2013). Factors such as pH and the ionic state of compounds, chemical complexation, hydrogen bonding, electrostatic interaction, van der Waals forces and non-specific forces between ionised molecules and organic matter can affect the sorption and subsequent desorption of both lipophilic and non-lipophilic compounds (O'Connor 1996; Horsing *et al.* 2011; Berthod *et al.* 2014; Morissette *et al.* 2015,). For compounds such as acidic pharmaceuticals, pH can be important to sorption processes as it can determine the ionic state of some compounds (Mompelat *et al.* 2009). Urase and Kikuta (2005) found that there was a linear relationship between partitioning coefficient (log K_p) and log K_{ow} for compounds without hydrophilic carboxylic functional groups (e.g. hormones, bisphenol-A, carbamazepine) but this was not observed for acidic pharmaceuticals (clofibric acid, gemfibrozil, ketoprofen, naproxen, diclofenac), which exist in the ionic form at neutral pH. Carballa *et al.* (2004) found that compounds that are more likely to remain in the aqueous phase (anti-inflammatories and antibiotics) may be less likely to be removed by adsorption, compared to compounds such as musks and estrogens, that may be more likely to be removed to sludge by adsorption (Carballa *et al.* 2004). Polar and ionic compounds show a tendency to display variable adsorption rates, depending on the characteristics of the sludge, compared to strongly hydrophobic compounds.

Morissette *et al.* (2015) found that sludges that had been pre-treated with alum to aid flocculation sorbed compounds less intensely than non-flocculated sludge. In general coagulation processes have been found to be inefficient in the removal of trace organic compounds (Ghernaout 2014). Coagulation can be impacted by the relative hydrophobicity of the compound, coagulant type, the water hardness and water pH. Changes in ion-exchange capacity, surface area of flocs, surface charge and particle size, and competition with inorganic ions present in effluent can impact the effectiveness of treatment with coagulants. This suggests that the composition of the wastewater can affect sorption processes. da Silva *et al.* (2011) found that log K_{ow}, does not give a straightforward linear relationship for partitioning when normalised to organic carbon content in municipal wastewater suggesting that the organic matter content may be important to sorption processes. The study by Morissette *et al.* (2015) also indicated a relationship between higher organic carbon content in sludges and greater sorption.

2.2.2.1.3 Complexity of sorption processes and influence of DOC

Models have been developed to predict phase distribution of POPs between aqueous and solid phases (Persson 2003). Barret *et al.* (2010) describe municipal wastewater sludges as a three-compartment matrix with compounds partitioning into the solid phase, but also sorbed to dissolved and colloidal matter in the aqueous phase (Barret *et al.* 2010). Partitioning of compounds between solid and liquid phases has been found to be influenced by the particulate organic matter, inorganic particulate and dissolved organic matter concentrations (Persson 2003). Concentrations of some compounds in sludges (e.g. dioxins (PCDDs), furans (PCDFs), naphthalenes (PCNs)) were found to correlate better with inorganic particulate content than particulate organic matter. Higher solids concentrations can be linked to a significant decrease in partitioning due to the presence of materials such as non-settling microparticles and dissolved organic carbon binding solutes that may keep organic pollutants in the aqueous phase as opposed to settling into sludges (Katsoyiannis and Samara 2005). Partitioning of highly chlorinated compounds such as PCBs showed a good negative linear relationship with dissolved organic carbon DOC (as DOC increased PCB sorption decreased). DOC can act as a microscopic pseudo-solvent, carrying micropollutants through treated effluent in the dissolved phase (Katsoyiannis and Samara 2005 & 2007; Baker *et al.* 2012; McPhedran *et al.* 2013). This suggests that the relationship between sorption of pollutants in sludge and organic carbon content in the wastewater matrix may differ by the relative proportion of organic carbon in the dissolved phase. DOC can potentially facilitate the movement of pollutants through a treatment plant, reducing their susceptibility to degradation, resulting in lower removal percentages during the treatment process. However, removal of biodegradable DOC in the dissolved phase in later stages of treatment could affect the relative level of partitioning of some POPs to sludges.

The importance of DOC to the movement of organic pollutants through a wastewater treatment system implies that distribution patterns of compounds between dissolved and adsorbed phases may vary by treatment stages (Katsoyiannis and Samara 2005). Although it may be predicted that partitioning of hydrophobic compounds occurs more significantly at primary settlement stages, an increased quantity of dissolved organic matter and inorganic particulate could result in hydrophilic compounds partitioning to a greater degree, or preventing some hydrophobic compounds from partitioning to sludges during primary treatment, and instead moving as part of the dissolved fraction towards later treatment stages. This implies that municipal wastewater sludges collected at different stages of the wastewater treatment process may vary depending on many factors including the pH, DOC, HRT, and presence of other materials such as flocculants.

2.2.2.2 Biodegradation

Different groups of microbes are responsible for degradation at different stages of the treatment process but the main mechanisms of compound removal by biodegradation can include processes of oxidation, hydrolysis, and demethylation (Miege *et al.* 2009; Baker and Kasprzyk-Hordern 2013). For highly toxic compounds, such as PCBs and pesticides, reductive dehalogenation is the most significant degradative pathway, but this may be affected by the microbial consortia that are present in a treatment system (Rogers 1996). In most models, the biodegradation rate of compounds is assumed to follow first order kinetics (assuming constant temperature) (Field *et al.* 1992; Chen *et al.* 2009; Sadef *et al.* 2014; Xu *et al.* 2016). In general, compounds with highly branched hydrocarbon chains and saturated aliphatic compounds are less likely to be biodegraded than unbranched and unsaturated compounds (Rogers 1996). Differences in functional groups or presence of sugar-type (moieties) could make compounds more biodegradable. Microorganisms tend to show a preference for easily biodegradable substrates such as glucose and peptone, however in the absence of these, will act on other types of organic compounds (Onesios *et al.* 2009; Urase and Kikuta 2005). Compounds such as aspirin, caffeine, ibuprofen and others may be degraded by microbes before less biodegradable ones such as sulfamethoxazole or carbamazepine (Rogers 1996). Halogenation of compounds (e.g. fluorination, chlorination, bromination) can make a compound less biodegradable with many of the most persistent compounds being halogenated organics (e.g. PCBs, PCDDs, PCDFs) (Wang and Jones 1994; Onesios *et al.* 2009). In general, higher molecular weight and more complex compounds, with non-biodegradable functional groups (e.g. halogens) also represent those that are most resistant to degradation.

Given the complexity of many POPs, first order kinetics may not accurately describe biodegradation rates for all compounds, and laboratory experiments may not adequately describe kinetics in full-scale treatment plants or the environment (Nyholm *et al.* 1996; Xu *et al.* 2016). In observing organic pollutant removal, the initial assumption of first order degradation may be due to both an initial rapid decline due to volatilisation. Non-linear behaviour however may be due to compound specific characteristics, competition for other organic carbon sources amongst the microbial consortia and transitions between dissolved and sorbed phases affecting bioavailability and bioaccessibility (Barret *et al.* 2010; Subedi *et al.* 2014). Compounds that are located in the aqueous phase of activated sludge may be the most bioavailable compounds. Other compounds may be bioaccessible (e.g. sorbed to particles) but not bioavailable until they transfer back to the aqueous phase during the treatment process. Highly hydrophobic compounds bound to sludge solids are less likely to be acted on by microbial processes.

Removal by biological processes may be affected by the prior exposure and adaptation of microorganisms to POPs, and the source water inoculum potentially impacting the biodegradation or biotransformation processes that occur (Onesios *et al.* 2009). Some authors have considered the role of nitrification and the role of ammonia oxidizing bacteria (AOB) on the fate of POPs (Fernandez-Fontaina *et al.* 2012; Maeng *et al.* 2013). A review by Xu *et al.* (2016) finds that higher rates of biodegradation were found in WWPTs with nitrification compared to those without nitrification, due to co-metabolism of organic compounds by ammonia oxidizing bacteria. This co-metabolic pathway may also result in different biodegradation pathways for some compounds such as variation in the degradation or transformation by-products. This includes oxidation and hydroxylation reactions that may not be present during normal metabolic biodegradation but are facilitated by the ammonia monooxygenase enzyme produced by AOB. Whether a plant is nitrifying or not does not appear to affect the sorption process, therefore differences in nitrification are only predicted to impact the biological removal stages (e.g. secondary treatment stages) (Fernandez-Fontaina *et al.* 2012).

The presence of toxic micropollutants may affect the efficiency of some microbes in the treatment system. While Abegglen *et al.* (2009) found no adverse effects from 0.5 $mg \cdot L^{-1}$ sulfamethoxazole, sulfapyridine, trimethoprim, clarithromycin and roxithromycin on nitrification or denitrifying capacity of the treatment system, Fountoulakis *et al.* (2004) found inhibitory concentrations of pharmaceuticals on a methanogenic consortium from a bioreactor continuously operated with an HRT of 20 d. The study also found that there was a direct correlation between the level of the pharmaceuticals affinity to adsorption to sludges and the level of inhibition which may be as a result of increased tendency for pollutants to adsorb to cell membranes.

2.2.2.3 Abiotic degradation

While sorption and biodegradation are the dominant removal processes in a WWTP, additional processes may also contribute to organic compound removal. The main abiotic processes include volatilisation and chemical degradation.

Volatilisation of organic compounds such as PPCPs as well as other persistent compounds such as pesticides is related to both the Henry's law constant and operational parameters such as aeration or temperature (Rogers 1996). Most pharmaceuticals have low Henry's law constants, therefore loss by volatilisation is negligible (Lin *et al.* 2010) however some compounds (e.g. fragrances/musks) may be slightly volatile (Miege *et al.* 2009). Compounds with a high Henry’s Law constant can be volatilised, particularly in treatment systems where forced air is used (e.g. systems with extended aeration such as nitrifying systems), causing some compounds to be volatilised to air or distributed into the dissolved phase (e.g. organochlorides such as α-Hexachlorocyclohexane) (Katsoyiannis and Samara 2005).

Chemical removal processes include hydrolysis, photolysis and oxidation, with oxidation being the dominant abiotic process. Oxidation of organic compounds can lead to the formation of hydroxylated aromatic compounds, followed by degradation of the benzene ring by ring fission (Wang and Jones 1994). Advanced technologies such as ozonation can enhance oxidation, and in conjunction with UV has been found to be an effective treatment option for some PPCPs and could be an effective treatment option for sludges (Lin *et al.* 2010). However, studies have shown that the type of chemical and the chemical structure (e.g. enantiomers) can affect the rate of degradation and therefore, effectiveness of treatment may vary by the types of structures present (Buser *et al.* 1999).

2.2.3 Operational and process parameters

Operational conditions (e.g. hydraulic retention time, solids retention time, aeration) and characteristics such as temperature, dilution rate and pH may affect removal rates and be more important to overall removal than process type (Gardner *et al.* 2013; Samaras *et al.* 2013; Luo *et al.* 2014). The effects of key operational conditions on organic compound removal are discussed briefly.

2.2.3.1 Hydraulic retention time (HRT)

Many authors have studied the effect of retention time on organic pollutant removal in wastewater effluent, which may be related to increased sorption and biological removal (Abegglen *et al.* 2009; da Silva *et al.* 2011; Barbosa *et al.* 2016). In a study of six WWTP in Spain, all with different retention times, plants with HRT of less than 12 h, experienced poor or no removal from influent of most compounds studied. For plants with HRT >25 h, removal was significantly improved (da Silva *et al.* (2011). In general, an increased HRT appears to be associated with higher removal rates of POPs in effluent. As previously discussed, the study by Morissette *et al.* (2015) also showed that an increased HRT enhanced the removal of less hydrophobic compounds by sorption processes. There has been limited study on the effect of HRT on concentrations of a wide range of POPs in biosolids.

2.2.3.2 Solids retention time (SRT)

The sludge age or solids retention time (SRT) has been found to influence the removal of organic compounds in WWTP effluent. The average SRT in WWTP varies from a few days to 30 d depending on the treatment process (Kinney *et al.* 2006). Clara *et al.* (2005) found that the degradation of bisphenol-A, ibuprofen, bezafibrate and natural estrogens showed a strong correlation with SRT, with maximum removal achieved by about 10 d, however other compounds showed an inconsistent relationship between removal and SRT (e.g. diclofenac, 17A ethinylestadiole), and the removal rate of others was not affected by SRT (e.g.

carbamazepine). Jelic *et al.* (2011) found that in a comparison of three WWTP, the two plants with the longest SRT offered better removal for the majority of compounds compared to the other plant. Batt *et al.* (2007) found that SRT positively influenced the reduction of antibiotics (e.g. ciprofloxacin, sulfamethoxazole, tetracycline, trimethoprim).

The mechanism by which SRT may affect organic compound removal could be due to effects on sorption processes as well as biodegradation processes (Miege *et al.* 2009). Kinney *et al.* (2006) predicted that the sorption of hydrophilic polar compounds in municipal wastewater sludges may have been linked to extended SRT, due to greater contact time with solids. For biodegradation processes, a high SRT allows for the growth of slower growing bacteria allowing for a more diverse biological community to develop, with wider range of physiological actions than systems operating with a low SRT (Luo *et al.* 2014). As sludge age increases, the biological component of sludge also increases in mass as solids are consumed, therefore the relative proportion of microbial biomass to organic solids increases with age (Abegglen *et al.* 2009). Extended SRT facilitates the growth of slower-growing nitrifying bacteria. Exposure to nitrifying bacteria may enhance some biodegradation pathways and longer SRT can keep sludge in contact with the microbial degraders for an extended period (Fernandez-Fontaina *et al.* 2012; Kruglova *et al.* 2014; Luo *et al.* 2014; Xu *et al.* 2016). The composition of the sludge however may impact how effectively degraders will remove organic pollutants. As readily biodegradable sources of organic matter are depleted, microbes may seek out alternative sources of carbon and energy and discrepancies in biodegradation studies indicates that the presence of other organic carbon-based substances has an impact on co-metabolism (Onesios *et al.* 2009; Semblante *et al.* 2015).

2.2.3.3 Temperature

Fluctuations in temperature can affect biological processes and hence biological removal of organic compounds (Miege *et al.* 2009). Temperature can affect nitrification (EPA 1974) and has also been shown to affect sludge growth, with an estimate that every 10 °C increase in temperature leads to a duplication of microbial activity (Clara *et al.* 2005). Barret *et al.* (2012) found that in laboratory scale aerobic reactors fed with organic pollutant contaminated sludges, enhancing temperature led to faster degradation of higher chlorinated PCBs.

2.2.3.4 pH

pH has been shown to have various effects on organic pollutant removal. pH has been shown to affect nitrification processes, and hence biodegradation rates (EPA 1974). pH has also been shown to affect the partitioning between aqueous and solid compartments of municipal wastewater sludge due to effects of ionic phase of compounds. Urase and Kikuta (2005) found that at neutral pH conditions, the water-sludge partition coefficients were low, and most pharmaceuticals remained in the aqueous phase. pH effects can depend on ionic strength with the effect being highest at low ionic strength, but not significant at higher ionic strength. Ionic strength can affect the adsorption of organic compounds onto a sorbent, either increasing or decreasing adsorption (Kyriakopoulos *et al.* 2006). The effect of pH can be compound specific and associated with the acid dissociation constant (pKa) of a compound. For example, for compounds with phenolic hydroxyl groups, dissociation of these groups can occur at pH above the pKa, causing the compound to become negatively charged and resist adsorption to similarly charged binding sites, reducing sorption capacity. Giudice and Young (2011) found that differences in sorption of triclosan and triclocarban in soils (pH 8.0) was attributable to their relative pKas, with triclosan (pKa 8.1) being primarily in the ionic phase around pH 8.0, and resistant to sorption compared to triclocarban (pKa 12.7), which is predominantly in the neutral form and less mobile. This is despite both compounds having similar log K_{ow} (4.8 and 4.9 respectively), which might suggest similar sorption behaviours.

2.2.3.5 Organic loading rate

The organic loading rate (OLR) has been identified as important to removal of organic compounds in wastewater treatment processes. Kruglova *et al.* (2014) found that the greater the OLR, the lower the degradation rate of pharmaceutical compounds such as ibuprofen in WWTP. This finding agrees with other authors, who find that availability of readily biodegradable organic carbon sources decreases the biodegradation rate of more resistant compounds (Urase and Kikuta 2005; Onesios *et al.* 2009). OLR could also affect the concentration of dissolved organic carbon, and the transfer of pollutants sorbed to DOC through the treatment process. Higher

DOC may reduce sorption of some pollutants, which may be more likely to remain in the aqueous phase of effluent rather than removed in solids.

2.2.4 Generalising POP removal processes at various wastewater treatment stages

The discussion of the various factors influencing removal of POPs in WWTP presented above emphasises the complexity of predicting removal rates at various treatment stages. Some compounds may be fully mineralised to CO_2 and water whereas others may remain unchanged or only partially degraded, transported in the aqueous phase to effluent or partitioned to sludge. The following sections examine how POP removal may vary at different treatment stages within a WWTP, given these influences discussed.

2.2.4.1 Primary treatment

Primary treatment reduces total organic carbon (TOC), biological oxygen demand (BOD) and chemical oxygen demand (COD) in WWTP influent by removing a major proportion of the organic load through settlement or clarification. Primary treatment is a passive process, relying on basic settlement or dissolved air floatation to clarify effluent as well as separate fats and greases. Relatively unaltered solids are collected as a concentrated sludge. The principle removal mechanism for organic contaminants is associated with the physical interaction between pollutants and the solid particles, or removal by sorption onto solids, and eventual sedimentation. Hydrophobic compounds may be removed more extensively than water soluble compounds as discussed previously (Gardner *et al.* 2013). Some loss by volatilisation may also occur for highly volatile compounds (Katsoyiannis and Samara 2005). The proportion of fats and greases in the wastewater may affect the removal of some very lipophilic compounds. This can include compounds such as fragrances and hormones (e.g. 17b-estradiol) (Carballa *et al.* 2004). Operational parameters such as HRT and pH are the most likely to affect sorption processes in primary treatment stages. Carballa *et al.* (2004) suggest that enhanced primary treatment could assist in reducing substances earlier by allowing greater opportunity for adsorption to occur by increasing residence time in clarification tanks. In addition, pH may be important for determining the ionic state of some polar or hydrophilic compounds, thus affecting their potential for settlement into sludge solids at the primary stage. Although not a biological treatment stage, some biotransformation may occur during primary treatment due to action of microbes. Gardner *et al.* (2013) found that some compounds did not decrease following primary treatment, and in fact increased (e.g. steroid estrogens (E1, EE2)) possibly due to biotransformation processes.

2.2.4.2 Secondary treatment

Various configurations of secondary treatment are used in modern WWTP. The principle of the treatment is aeration of the sludge (activated sludge, or AS) to encourage growth of the aerobic microbial community that consume organic matter and increase the potential for compound removal. Conventional activated sludge (CAS) is probably the most commonly used system, but other configurations such as membrane bioreactors (MBR), cyclic activated sludge systems (CASS) sequencing batch reactors (SBR) or trickling filters (TF) are used, amongst others. The difference between systems can be generalised by the method of aeration, the process of liquid/solid separation, and the length of time that sludge is held within the system however in nearly all secondary treatment, organic contaminants will be removed through a combination of biodegradation, biotransformation, sorption to the activated sludge and additional volatilisation due to aeration forcing air through the sludge and effluent (Byrns 2001; Katsoyiannis and Samara 2005).

Secondary treatment has different impacts on different compound types and several researchers have assessed the effectiveness of secondary treatment systems on the removal of organic compounds from municipal wastewater influent. In general, the literature finds high variability in the removal efficiencies of various organic compounds undergoing secondary treatment (Katsoyiannis and Samara 2005; Batt *et al.* 2007; Miege *et al.* 2009; Gros *et al.* 2010; Baker and Kasprzyk-Hordern 2013; Gardner *et al.* 2013; Blair *et al.* 2015). For studies that compared CAS, MBR and TF processes, the general findings were that minimal difference was observed in removal rates for CAS and MBR systems (Clara *et al.* 2005; Oppenheimer *et al.* 2007), however both of these systems achieved higher removals overall compared to TF (Baker and Kasprzyk-Hordern 2013; Gardner *et al.* 2013). The findings of the literature suggest that plants that have extended aeration (nitrogen removal) may reduce pollutants better than plants designed for BOD removal only (Clara *et al.*

2005; Miege *et al.* 2009). The process type and compound type may be less important than some operational factors to the removal rates. Biological degradation or transformation processes are the most important removal mechanisms for micropollutants during secondary treatment, therefore factors that affect biological processes should also affect removal rates (Abegglen *et al.* 2009; Gros *et al.* 2010; Barbosa *et al.* 2016). Removal efficiency of biodegradable compounds has been shown to increase with increasing HRT and SRT; however, for non-biodegradable compounds such as carbamazepine, removal rates do not improve with longer retention times (Gros *et al.* 2010).

2.2.4.3 Other types of treatment

The effects of tertiary treatment on organic pollutant removal from effluent will not be evaluated in this study, due to the plants selected being limited to primary and secondary treatment only. However, additional effluent treatments may be applied in some systems such as coagulation-flocculation processes to provide additional clarification of the effluent. As discussed previously, coagulation/flocculation processes are observed to provide minimal removal of many organic compounds, and the best removal rates (20-50%) are observed for compounds with high K_{ow}, for which removal is more likely at primary treatment stages (Luo *et al.* 2014). Verlicchi *et al.* (2015) found that additional treatments such as addition of activated carbon showed good results for removal across groups of drugs from hospital wastewater, at levels greater than 90% but removal via coagulation/flocculation showed consistently poor results (<20-40%). Tertiary treatments such as these are unlikely to produce large volumes of biosolids compared to primary and secondary stages.

Although this study did not assess sludges from septic tanks and small onsite wastewater treatments systems (OWTS), it is worth considering factors affecting POP loading in these systems. In OWTS, sludges will typically be transferred to conventional treatment plants when tanks are de-sludged and combined with sludges from larger plants, therefore they will form a proportion of biosolids in larger WWTP. Few studies have assessed the fate of POPs in septic tank systems (Petrie *et al.* 2015; Subedi *et al.* 2015), and hence limited insight into the effect of processes occurring in septic tanks on POP levels in effluents are known. However, two key features of septic tanks may be important to organic compound removal. These include the extended HRT and SRT in OWTS, and the anaerobic conditions that may establish in the bottom sludge. In general, aerobic conditions are always better than anaerobic for reduction of organic compounds but where anaerobic conditions develop in a septic tank, some compounds may be better removed. A high sludge age may result in reduced biological activity in onsite systems, but potentially more specialised and adapted microorganisms (Abegglen *et al.* 2009). There are also differences in the number and source inputs to the system and the loading of POPs may be much more variable compared to larger systems where influent variability is averaged out over a larger population (Abegglen *et al.* 2009). Influent into large treatment plants may also differ based on dilution effects of stormwater that may be included in centralised plants, but typically not in OWTS.

Finally, a consideration of common sludge treatment processes following wastewater treatment is needed. Common treatments include dewatering, anaerobic digestion (AD) and thermal treatment. Some authors have also assessed the effect of dewatering processes on micropollutant concentrations (Mailler *et al.* 2014; Marti and Batista 2014) but find that these processes typically do not influence pollutant concentration in the sludge. The effect of additional treatment by AD varies depending on the compound and some compounds will only biodegrade under anaerobic conditions (Chaney *et al.* 1996; Abegglen *et al.* 2009). Barret *et al.* (2012) found that lighter polycyclic aromatic hydrocarbons (PAHs) are better degraded than heavier ones under anaerobic conditions. AD has been found to provide very high removal efficiency of sulfamethoxazole (>80%), 17a-estradiol, and high removal (>60%) of galaxolide, tonalide and diclofenac, medium removal of diazepam (50%) and ibuprofen (20-50%) (Carballa *et al.* 2007). However not all compounds are effectively treated by AD. Marti and Batista (2014) found that AD processes increased the concentrations of estrogens, due to destruction of solids, and desorption of estrogens.

2.2.5 Removal efficiency by compound type

Overall there is agreement in the literature that treatment process stage and operational parameters may impact organic compound removal and partitioning to sludges, but it is difficult to generalise the types of effects across the wide array of compound types. Luo *et al.* (2014) reviewed the effect of treatment type on organic micropollutants with the findings summarised in Table 2-2. Generalising treatment effects across the categories of pharmaceuticals and industrial chemicals is not always straightforward. For example, Pomies *et al.* (2014) showed that some pharmaceuticals are eliminated well by biodegradation in wastewater treatment processes (e.g. >90% removal for atenolol, ibuprofen and paracetamol) but poorly removed by adsorption to solids. Some pharmaceuticals, such as amitriptyline and fluoxetine may be relatively well removed by either process (e.g. 70-90% removal), whereas others are removed to a lesser degree or not at all. A review by Onesios *et al.* (2009) found that drawing generalisations on the impact of treatment processes on therapeutic classes of compounds (e.g. antibiotics, anti-cancer drugs, anti-inflammatories etc.) was not possible, with wide ranging removal rates within drug classes apart from antiseptic compounds. This was also observed by Jelic *et al.* (2011), who found that characterising the effect of wastewater treatment processes by contaminant type was not possible as variation within and between therapeutic classes of pharmaceuticals was observed. Only a few substances are found to be consistently biodegradable (e.g. aspirin, acetaminophen, caffeine) or consistently non-biodegradable (e.g. carbamazepine, codeine phosphate) across treatment plant types and operational conditions (Richardson and Bowron 1985; Onesios *et al.* 2009; Maeng *et al.* 2013).

Table 2-2 Summary of treatment effects of various wastewater treatment processes on pharmaceuticals, personal care products (PCP), hormones and industrial chemicals in wastewater effluent (adapted from Luo *et al.* 2014)

Treatment process	**Removal efficiency**				**Influencing factors**							
	Pharmaceuticals	PCP	Steroid hormones	Industrial chemicals	pH	dose	wastewater composition	HRT	SRT	organic loading	redox conditions	other
Coagulation	L-M	M-H	L	L-H	✓	✓	✓					
Ozonation & Advanced oxidation processes (AOP)	M-H	M-H	H	M-H	✓	✓	✓					interfering ions
Activated sludge	L-H	M-H	M-H	L-H				✓	✓	✓	✓	
MBR	L-H	M-H	H	M-H				✓	✓	✓	✓	
Attached growth/TF	L-H	M-H	M-H	M-H				✓		✓	✓	

L=low, M=medium, H=high; MBR = membrane bioreactor, TF = trickling filter

2.3 Conclusion

The widespread release of persistent organic pollutants into the environment has been shown to have negative impacts on the environment and human health. Organic pollutants are being released in greater numbers and variety than ever before, and are now ubiquitous across environmental compartments of air, water and land and detected in human blood and tissues. WWTP represent an important pathway for many POPs to reach the environment, and therefore greater understanding of how treatment systems impact on concentrations of contaminants in municipal wastewater sludges and effluents is essential to managing environmental and human health risks.

The literature indicates that POP removal is variable across treatment types and the interventions required to adapt treatment systems for removal of emerging compounds is of limited consideration for most operators unless operating under a specific regulatory consent condition. In general, it can be stated that sorption to solids and dissolved organic and colloidal matter are the processes that dominate primary treatment stages and may account for greater removal of hydrophobic compounds. These compounds are less bioavailable, and therefore are more likely to remain in the sludge relatively unchanged. Operational parameters such as pH can affect sorption processes by affecting the dominant ionic form, particularly for compounds such as acidic pharmaceuticals (Urase and Kikuta 2005). In addition, the concentration of TOC, and dissolved organic and colloidal matter can increase the potential for organic compounds to bind to these and either settle into primary sludges or be carried through to secondary treatment as part of the dissolved and colloidal matter. The literature to date provides only limited consideration of how treatment plant type, and operational parameters may impact the level of POPs that accumulate in WWTP sludges.

Subsequent treatment stages may remove POPs by a range of processes but removal in most secondary treatment stages will be dominated by biodegradation process. During secondary clarification, organic compounds will sorb to remaining particulate matter, including surfaces of bacterial cells. The presence of readily available organic carbon in secondary treatment stages may reduce the biodegradation rate of less biodegradable organic compounds, but in conditions of low availability of readily degraded organic carbon sources, microbes may degrade more non-biodegradable sources of organic carbon. Nitrification processes have been shown to enhance biodegradation processes, by increasing the contact time between the active microbes, and organic compounds, as well as introducing additional enzymes that may assist in additional degradation steps.

The effectiveness of pollutant removal from effluent is pertinent to the study of possible pollutants in sludges. Removal by biodegradation suggests a reduction in the possible quantity of pollutants that will be sorbed to sludges. However, processes that affect sorption will influence the relative quantities of materials accumulating in sludges, potentially unexposed to biodegradation processes. While the literature indicates widespread study of POPs in aqueous phase, there is much less study of POPs retained in the wastewater solids. The literature has provided indications of the types of compounds that may be in these solids however many variables have been identified that can affect sorption processes, biodegradation pathways and abiotic removal. This variability presents a challenge in identifying treatment processes that could be adapted for organic pollutant removal. Additional research is needed to understand the eventual fate of POPs in this matrix and the implications for land application processes. This implies a need for better understanding of both the composition of biosolids as well as the potential for POP to migrate from sludges applied to land (Carballa *et al.* 2004; Baker and Kasprzyk-Hordern 2013).

CHAPTER 3 LITERATURE REVIEW: MUNICIPAL WASTEWATER TREATMENT SLUDGES AND THE ENVIRONMENT

3.1 Introduction

As discussed in Chapter, 2, wastewater treatment sludges can be a sink for POPs from human waste, disposal of pharmaceuticals, industrial and hospital effluents, and run-off from roads and surfaces. The aim of this chapter is to provide a more detailed definition of sludges and biosolids, to examine the current disposal practices for these materials around the world and explore evidence of organic pollutant transfer from sludges and biosolids into the environment. This chapter also examines the approaches to managing risk from environmental transfer of POPs. The chapter proposes an alternative approach for assessing risk associated with the transfer of POPs into the environment from a municipal wastewater treatment sludge or biosolid matrix.

3.2 Municipal wastewater treatment sludges and biosolids: definitions and current disposal practices

Influent to WWTP can include a combination of sources including domestic households, industrial operations, hospitals, storm water or drainage sources. Through the wastewater treatment process, solids from these sources will be separated from aqueous effluent, producing sludges that contain a mixture of particulates, microbial biomass and sorbed pollutants as well as chemicals (e.g. alum, ferric chloride, polymers, pH treatments) added during the treatment process. At the end of the wastewater treatment process, sludges are collected and typically dewatered in presses or centrifuges, producing a substance that can be disposed of (e.g. to landfill) or further treated by drying, composting, or other process intended to reduce moisture content, or lime addition to reduce odour and reduce pathogen count. The end-product of this process can be materials such as sludge cake, sludge pellets, or other form of dewatered sludge, collectively referred to in this study as biosolids. The primary difference between sludges and biosolids is the additional treatment the material undergoes to reduce pathogens and odour (e.g. drying, lime treatment) (Eljarrat *et al.* 2008). Sludge treatment is not designed for reduction of organic or inorganic contaminants, but typically only for the reduction of odour and pathogens. The following sections examines the composition, disposal and regulation of municipal wastewater treatment sludges, using the term “biosolids” to collectively refer to these materials in the form they are applied to land, which at a minimum typically includes a dewatering process.

3.2.1 Biosolids composition

Compared to the soils they may be applied to, biosolids have a higher biomass content, a lower humic acid content and contain microbial flocs (Dizer *et al.* 2002) and where they have been treated with lime, have a higher buffer capacity. Biosolids are typically rich in nutrients such as phosphorus and can provide an alternative to commercial fertilisers offering possible economic and environmental benefits as global reserves of phosphorus are depleted (Cordell *et al.* 2009). The application of biosolids to land can increase water retention, cation exchange capacity and can help to retain essential plant nutrients (Singh and Agrawal 2008). This is primarily due to their high quantity of organic matter (OM) content, which can range from 20-43% to 40-80% by dry weight (dw) (Singh and Agrawal 2008; Carbonell *et al.* 2009). Gielen *et al.* 2011 characterised the organic matter in biosolids to be almost two-thirds protein and approximately one quarter carbohydrates, with cellulose (e.g. toilet paper) the predominant carbohydrate, and the remaining fraction being fatty acids and aromatics. Torri and Alberti (2012) found that about 13% of the organic matter in biosolids is polar, with about half of this comprised of fatty acids (vegetable oils, animal fats, human faecal fatty acids) and steroids, and about 14% as aliphatic compounds (n-alkanes). Torri *et al.* (2003) found that 29-45% of the organic carbon in biosolids spread to land was recalcitrant. The organic compounds in the recalcitrant fraction included fatty-acids, n-alkanes, steroids, and POPs below levels of detection, predicted to be largely comprised of stable sterols. This recalcitrant fraction, however, may also have contained a wide array of unknown persistent organic compounds that were not assessed by Torri *et al.* (2003).

3.2.2 Biosolid disposal

Disposal options for biosolids include landfill, incineration, composting, land application or dumping at sea (McClellan and Halden 2010). Dumping at sea was initially a widespread disposal option for municipal wastewater sludges globally. In the latter part of the twentieth century widespread media attention and public criticism, particularly in developed countries, of dumping at sea was observed. This practice was blamed as the cause of so-called “dead zones” in the sea, caused by the mixture of toxic compounds found in sludges (Weis 1988). High profile cases of beach pollution in locations in New York and New Jersey in the late 1980’s led to the eventual ban on dumping of sewage sludge at sea in the United States, resulting in land application becoming the primary disposal option for approximately 60% of the 5.6 million dry tons of biosolids produced in the U.S. per year (NRC 2002; McClellan and Halden 2010). Increasingly, land application of biosolids is becoming the preferred end use in place of disposal to landfill or incineration around the world as shown in Figure 3-1 (BC Government 2019). In the EU, it is estimated that the total sludge generated from urban WWTP in the EU increased from 5.5 to 10 million tonnes dry matter between 1992 and 2008 with over half of this material being spread as biosolids to land in agricultural or horticultural applications (50-65%), and the remaining being landfilled or incinerated (Carbonell *et al.* 2009).

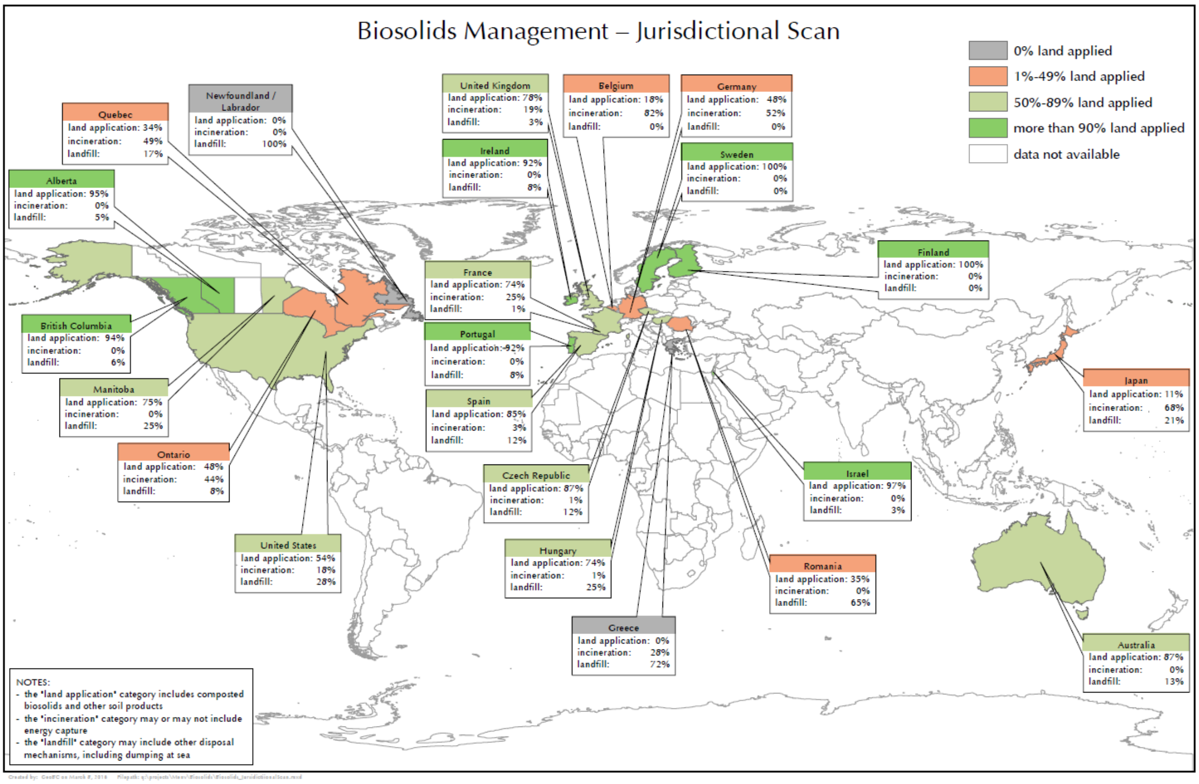


Figure 3-1 Fate of biosolids around the world (BC Government 2019)

It is estimated that 240 kg dw of biosolids are produced per million litres (L) of treated wastewater (Kinney *et al.* 2006). Based on global estimates of wastewater production of 3.3 x 10^{14} L of wastewater annually, an estimated 7.92 x 10^{13} tonnes of biosolids are produced annually each year (Mateo-Sagasta *et al.* 2015). Land application of some of this massive quantity of waste provides an opportunity for nutrient recycling, returning organic matter to soils, and eliminating the need for landfilling large quantities of solid materials that could contribute to the generation of greenhouse gases. However, nutrient recycling back to land in the form of biosolids brings an accompanying matrix of organic matter and pollutants for which relatively little assessment of the potential environmental consequences has been undertaken.

3.2.3 Global regulation of land application of biosolids

It is ironic that the public perception of ocean dumping as the cause of “dead zones” in the sea was seen as a less favourable option than application of sludges on land. However up to the point of change in management practices for wastewater sludges, relatively little study had taken place assessing the potential for pollutant transfer from biosolids to terrestrial environments, including crops and freshwater bodies. As the practice of land application of biosolids increased, early regulation was driven by public health concerns about spread of infection and disease, and public nuisance concerns such as odour (Chaney *et al.* 1996).

In the early 1990s, the first attempts to quantify risks to human health and the environment from the application of biosolids were undertaken. The most notable work was carried out by the United States Environmental Protection Agency (USEPA). The USEPA used a pathway risk assessment (PRA) approach to establish pathways of exposure for contaminants contained within municipal wastewater biosolids, to establish regulatory controls to protect Highly Exposed Individuals (HEIs). This approach found that pollutants in the source (biosolids) could reach the receptor (animals and humans) through contact with garden soils amended with biosolids, consumption of foods produced in biosolid amended soils including milk or meat from animals raised on biosolids amended soils, or through exposure to dust in homes in proximity of biosolid amended fields (Chaney *et al.* 1996). While this approach sought to protect HEIs, there were some limitations to its wider applicability. The focus on only HEIs eliminated the consideration of diffuse pollutant transfer into the wider environment and exposure of unintended receptors. The approach was based almost entirely on the risk of ingestion of biosolid particles from direct contact with biosolid, amended soils, and food rather than pollutant transfer from biosolids into food or water sources. The approach considered risk from a human health perspective only as opposed to ecological effects. This approach also excluded consideration of how relative risk changes over time, for example the potential for pollutant accumulation after successive applications of biosolids to soils, the duration over which risks are likely to be present, and the physicochemical processes involved in degradation or transformation of pollutants, changing the relative risk over time (Chaney *et al.* 1996). Table 2-1 in the previous chapter outlines several of the health risks and possible impacts on the environment associated with exposure to both pharmaceuticals and industrial additives or by-products, many of which would be considered emerging pollutants. This suggests that assessments of risks associated with pollutants in sewage sludges will need to be regularly reviewed over time, both to account for new pollutants that may be present but also to account for new research on the relative effects on humans and the environment.

The work by the USEPA led to the creation of the Part 503 Rule (*The Standards for the Use of Disposal of Sewage Sludge, Part 503 of Section 40 of the Code of Federal Regulations*), which sets out the environmental quality standards, operational standards and management practices for use and disposal of biosolids. Initially considered in this legislation, organics were deleted from the content of the Part 503 Rule in 1992 due to observational data indicating that many POPs of interest were found in insignificant concentration in biosolids or were no longer in production or had been banned, such as PCBs (Smith 2009; USEPA 2017). In 2002, the USEPA revisited a small number of organic compounds in biosolids (dioxins, and dioxin-like compounds) and carried out probabilistic risk assessments but concluded that no numerical limits or management practices were required to protect human health and the environment from these specific biosolids-borne contaminants. In 2006-2007, the National Sewage Sludge Survey (NSSS) was carried out by the USEPA to obtain updated data for nine pollutants of potential concern and screening data for new contaminants of emerging interest (USEPA 2009). Several antimicrobials and antibiotics were detected in the $mg \cdot kg^{-1}$ (dw) range, with prevalent contaminants including triclocarban, triclosan, ciprofloxacin and ofloxacin, followed by tetracycline antibiotics (McClellan and Halden 2010). Pollutants of potential concern were evaluated based on pollutants for which adequate data on human health benchmarks, and information on fate and transport in the environment were known. However, the results of these studies (NSSS) did not lead to any new regulatory measures for any of the contaminants or mixtures studied (McClellan and Halden 2010). Today, the only organic compounds regulated in sewage sludge under the Part 503 Rule are PCBs, where levels equal to greater than 50 $mg{\cdot}kg^{-1}$ dw total solids require the material to be treated under hazardous waste regulation.

The USEPA approach has been mirrored in many other countries with limited controls currently in place for organic contaminants in biosolids. Some countries such as New Zealand and some regions of Australia provide guidelines for organic contaminant concentrations in biosolids, typically limited to organochlorine pesticides and PCBs (NZWWA 2003, NSW EPA 2000, EPA Victoria 2004). However, other regions state that estimated concentrations of POPs are low or that treatment processes sufficiently reduce levels of organic contaminants below levels of concern (Environmental Protection Authority 1997). It is not clear on what basis some of these statements of “*below levels of concern*” are founded. In Canada, as in Australia, regulation varies by province, with some provinces adopting the USEPA Part 503 Rule, others adopting limits only for PCDDs and PCDFs, and most provinces specifying no limit on organic contaminant concentrations (CCME 2010). In the province of British Columbia for example, the management of biosolids as soil amendment, regulated under the Organic Matter Recycling Regulation (OMRR), sets criteria for pre-treatment of biosolids to reduce microbiological indicators (faecal coliforms) and limits for trace metals, but no requirements for organic pollutants (Metro Vancouver 2013).

In Europe, some countries have established criteria for pre-treatment and safe disposal of biosolids with restrictions on application near sensitive environments such as surface waters, groundwater protection areas or on sloping land. A few have established limits for a selection of organic pollutants (EC 2001). These include Austria with limits for PCDDs, PCDFs, PCBs, adsorbable organic halogen compounds (AOX) and PAHs; Belgium with limits for 30 organic compounds; Denmark with limits for linear alkyl sulphonates (LAS), di-2-(ethylhexyl)phthalate (DEHP), nonylphenolethoxylate (NPE), and PAH; France with limits on PCBs and PAH; Germany with limits on PCDDs, PCDFs, PCBs, AOX; and Sweden with limits on PCBs, NPE, PAH, Toluene (EC 2001). In Norway, regulation on Fertiliser Materials of Organic Origin allows for land application of biosolids and does not propose specific limits on POPs but requires land users to "*take reasonable actions to limit and prevent organic micro-pollutants that may cause harm to health or the environment*". This guidance is very generic and would be difficult to define what constitutes "reasonable actions" as well as "harm to health or the environment", particularly where chronic or ecosystem effects may occur.

In the UK, the *Sewage sludge on farmland: code of practice* (UK Government 2017) contains recommendations for safe application of biosolids (e.g. to reduce run-off into local water courses) but only limited mention of precautions for organic contaminants is included. This code of practice, along with a review of practice for the storage and application of sludge to land in Scotland in 2015 contributed to the development of a Biosolids Assurance Scheme (BAS) to ensure the quality of biosolids produced in the UK used for agricultural purposes. A not-for-profit company (Assured Biosolids Limited) was set up by eleven UK water and sewerage companies to develop and administer a UK-wide BAS. The scheme was launched in 2018, gaining accreditation under the UK accreditation scheme (UKAS) and sets criteria for biosolids processing and application based on assessment of hazards, namely levels of metals, pathogens and nutrients. The assurance scheme contains no mention of organic pollutants amongst the potential pollutants suggested for monitoring (Assured Biosolids Limited 2019). Prior studies have identified that UK biosolids contain various POPs, but relative risk to human health and environment from migration of these pollutants from biosolids has not been established (McIntyre and Lester 1984; Rogers *et al.* 1989; Smith and Riddell-Black 2007; Clarke and Smith 2011; WCA Environment 2014).

Despite the progress that has been made to promote global agreement on control of POPs in the environment through agreements such as the Stockholm Convention, and despite some of the pollutants listed in the convention being detected in biosolids, no globally comparable standards of sampling, monitoring, detection and quantification of POPs in biosolids have yet been established and these materials are not currently considered under the Stockholm Convention (Muir and Howard 2006; Fiedler *et al.* 2013). Without regulation of organic contaminants, there is currently little incentive for wastewater treatment operators to consider applying processes or additional treatments for the beneficial reduction of POPs in biosolids before application to land. Some countries have taken the position of banning application of biosolids from use on agricultural land altogether (e.g. Switzerland and the Netherlands). These countries have other outlets for biosolids (e.g. incineration) and there is a desire not to compete with an existing domestic fertiliser market (Netherlands) (LeBlanc *et al.* 2008). However, this strict stance removes the potential for beneficial uses of biosolid material to be realised, including return of nutrients and organic matter to soil.

The next sections provide a review of the evidence on pollutant transfer from biosolids into the environment, assessing occurrence and fate of many organic pollutants and key knowledge gaps needed to inform future management strategies for biosolids. The various methods currently employed to quantify risks and prioritise pollutants are discussed, including consideration of methods for assessing compound mixtures and limitations of current practices.

3.3 Occurrence and fate of POPs in biosolids.

The risks to human health and the environment from exposure to POPs in biosolids is associated with the diversity and quantity of toxins present as well as the potential for pollutants to move between environmental compartments. The longer a chemical persists in the environment, the greater the potential for exposure to occur and for repeated applications of low concentrations of persistent chemicals to accumulate. The following sections examine the evidence of the types and quantities of compounds present in biosolids, and their potential mobility between environmental compartments.

3.3.1 Occurrence of POPs in biosolids

Many studies have been carried out to attempt to quantify levels of a wide variety of organic pollutant levels in municipal wastewater treatment sludges and biosolids (McIntyre and Lester 1984; Rogers *et al.* 1989; Kinney *et al.* 2006; Smith and Riddell-Black 2007; Eljarrat *et al.* 2008; Diaz-Cruz *et al.* 2009; Edwards *et al.* 2010; Snyder *et al.* 2010; Clarke and Smith 2011; Jelic *et al.* 2011; Martin *et al.* 2012; Torri and Alberti 2012; Samaras *et al.* 2013; Venkatesan and Halden 2014; WCA

Environment 2014; Blair *et al.* 2015; Petrie *et al.* 2015; Semblante *et al.* 2015; Daughton 2016). These studies have typically used bespoke methods to isolate a select number of organic compounds using complex and time-consuming preparation methods. Biosolids represent a challenging matrix for organic compound analysis and multiple preparation steps are typically required to reduce matrix effects from biosolid constituents such as coagulants, surfactants, and various other substances before analysis can be performed. These steps can include sulphur removal, organic matter removal by acid treatment, or further steps to fractionate compounds using various adsorbents followed by extraction by Soxhlet solvent extraction or other methods (Eljarrat and Barcelo 2004; Diaz-Cruz *et al.* 2009). These techniques are necessary to isolate compounds and remove interferences in analytical equipment such as liquid chromatography (LC) coupled with mass spectrophotometry (MS) or LC-MS. Even with sophisticated clean-up procedures, results typically have a high level of uncertainty (Carrara *et al.* 2008). For example, the USEPA NSSS study produced results with high levels of uncertainty, with acceptance limits ranging from 5 to 200% for some organic compounds (e.g. pharmaceuticals, steroids and hormones) (USEPA 2009). Sample preparation can account for a significant amount of variability within methods; especially for trace compounds and existing methods may also not be selective enough to distinguish between the wide range of compounds (Tadeo *et al.* 2012). In addition, some common analytical approaches may not be suitable for some compound types, such as most pharmaceuticals that are not easily volatilised, and therefore not easily determined by methods such as gas chromatography (GC) (Diaz-Cruz *et al.* 2009).

Despite the uncertainties, and analytical complexity, many studies have identified many organic compounds to be concentrated in biosolids. Commonly identified compounds include pesticides (e.g. PCBs), industrial chemicals (e.g. organotins, PBDEs, polyfluorinated compounds (e.g. PFOS), polydimethylsiloxanes) and PPCPs (e.g. antibiotics, triclocarban, triclosan, synthetic musks, ibuprofen, naproxen) among many other (Clarke and Smith 2011; Martin *et al.* 2012; Sabourin *et al.* 2012; Samaras *et al.* 2013). Some of these compounds have been detected at measurable concentrations including triclosan at up to 1,500 $ng{\cdot}g^{-1}$ dw, PBDEs at over 50,000 $ng{\cdot}g^{-1}$ dw and PFOS up to more than 5,000 $ng{\cdot}g^{-1}$ dw (Eljarrat *et al.* 2016). One of the most comprehensive studies of POP levels in biosolids was carried out by

Venkatesan and Halden (2014) who carried out a survey of municipal wastewater sludge samples for 231 contaminants of emerging concern in samples originally collected during the 2001 USEPA NSSS. Of the compounds examined, 123 chemicals were positively detected in biosolids. The researchers looked for trends in the types of compounds detected in biosolids. For example, the relative abundance versus the log K_{ow}, or measure of lipophilicity, was assessed (Figure 3-2). The study found that most compounds (> 80%) were present at a medium level of abundance in biosolids (between 1-1000 $\mu g \cdot kg^{-1}$) with a relatively even distribution between compounds with a high and low log K_{ow}. More lipophilic substances were found at low abundance and a few of each type were found at high abundance. The study demonstrated that biosolids contain a diverse mixture of organic pollutants at medium abundance, both lipophilic and non-lipophilic, halogenated and non-halogenated.

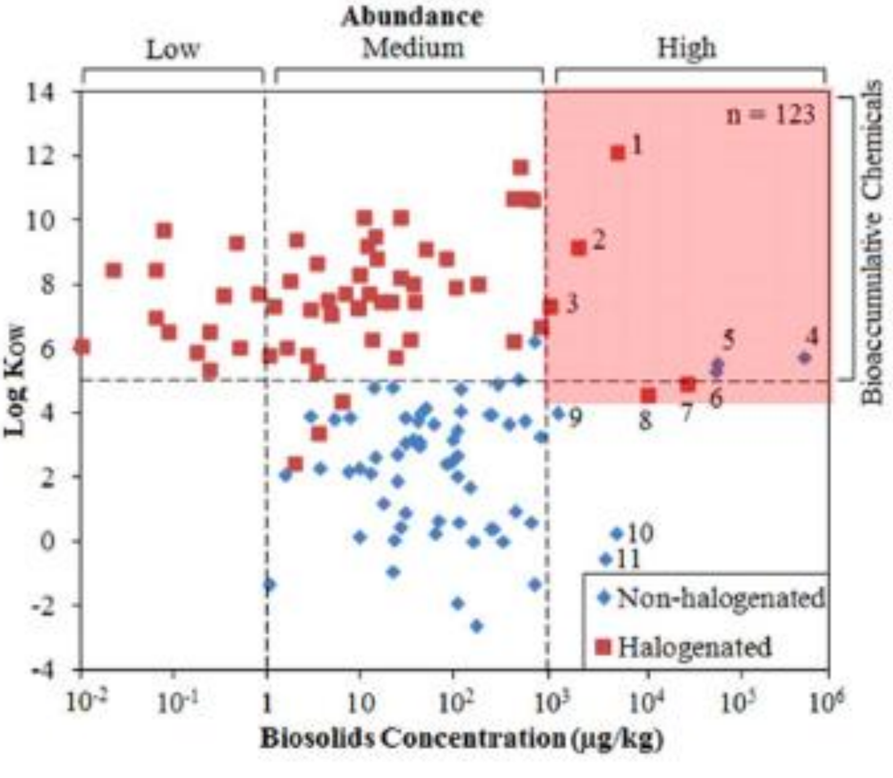


Figure 3-2 Classification of 123 chemicals detected in biosolids based on abundance in biosolids and on n-octanol water partition coefficient (K_{ow}). (Venkatesan *et al*. 2014)

The study by Venkatesan and Halden (2014) is significant because it highlights that biosolids do not accumulate only lipophilic compounds but contain a wide array of contaminants at appreciable concentrations, representing a diverse cocktail of potential pollutants. The analysis identifies the presence of lipophilic substances that may be associated with greater ease of uptake in tissue of living organisms, and thus have greater potential for toxic effects and bioaccumulation in living organisms, as well as non-lipophilic substances such as some pharmaceuticals have been specifically designed to pass cell membranes, which may also be relatively easily taken up by exposed cells (Halling-Sorensen *et al.* 1998). These compounds may also be potentially more readily desorbed under environmental conditions. The methodology of the study by Venkatesan and Halden (2014) exemplifies the complex analytical procedures that were required to isolate, identify and quantify the compounds of interest. The methods used included different extraction, digestion and clean-up techniques to isolate compounds, and advanced analytical equipment and methods to detect the compounds once isolated in a solvent extract or aqueous solution.

While useful in identifying pollutant partitioning behaviour and helping to quantify total burden of specific pollutants in a biosolid material, it should be noted that the application of solvent extraction techniques used in most of the studies mentioned have limitations. The use of solvents causes adsorptive bonds between POPs and sludge particles to be readily broken. In the environment, these bonds may not be broken, and hence some of the compounds may be immobile in the environment and have reduced bioavailability, presenting less exposure risk than substances that are mobile in more aqueous conditions such as after rainfall or flooding (Clara *et al.* 2010). Boxall *et al.* (2012) also note that many of the existing methods used to extract these non-extractable compounds cannot be done without significantly changing the compounds, and thus confusing the original chemical identities.

The literature clearly confirms that POPs and other organic compounds are present in biosolids sourced from municipal wastewater treatment sources. The literature also exposes several major challenges for environmental regulators related to establishing safe limits for biosolids spread to land. The level of effort required to process and analyse various organic pollutants is time consuming and expensive and presents a barrier in developed countries, but even more so in developing countries where resources are more limited. High levels of uncertainty in the methods also make establishing regulatory limits on individual compounds very difficult. Choosing which compounds to monitor or regulate is not straightforward, given the complex mixture of possible pollutants. Measuring a single compound or a select few does not provide an indication of the overall pollutant burden of the mixture of compounds present. No studies have been found that consider generic measures of organic pollutant burden in biosolids or relative pollutant mobility, assessing how POPs leach from biosolids.

3.3.2 Fate of biosolid derived POPs in the environment.

The review by Clarke and Smith (2011) on emerging organic pollutants identified some key findings relevant to understanding environmental risk from pollutant transfer from biosolids. The review found that some pollutants (i.e. antibiotics) were found to be more persistent in soil environments amended with biosolids than in aquatic environments at a scale of years (soils) versus days (aquatic environments). For other substances such as bisphenol-A (BPA), the review reported that persistence in soil was low, despite a wide range of measured concentrations in biosolids, as the compound was predicted to be rapidly removed via biodegradation processes. Organotins may persist in the soil in the order of months, but there was no consistent trend found for biosolids. Perfluorochemicals (PFCs) were found to be resistant to degradation in the environment due to chemical structure (strong carbon-fluorine bonds) but potentially mobilised by rainfall. Compounds such as triclosan and triclocarban (antimicrobials) were found to have variable solubility in water depending on pH, making these compounds likely to be sorbed during wastewater treatment (neutral to slightly basic pH), and subsequently released in the environment from biosolids (under slightly acidic pH). These materials were also found to be potentially persistent in the environment and bioaccumulative in algae, earthworms and snails.

In addition to the review by Clarke and Smith (2011) additional studies have documented the occurrence of POPs in biosolids, and the soils amended with them. Once in the environment, organic compounds have several possible fates (Halling-Sorensen *et al.* 1998):

- biodegraded partially or fully (to CO_2 and H_2O);
- not degraded and transported between environmental compartments;
- partitioned or sorbed onto some solid media or particulate matter;

- metabolised into a different form.

Torri *et al.* (2003) demonstrated that a large portion of the organic carbon within biosolids is readily biodegradable, with most of this material mineralised within the first 60 d after application to soil, however a significant fraction of persistent, non-biodegradable organic carbon remains. Others have also found evidence of non-biodegradable organic compounds remaining after sludge application. Eljarrat *et al.* (2008) found that PBDEs in biosolid amended soils were present at concentrations of 21-690 $ng{\cdot}g^{-1}$ dw with an indication that the compounds may be accumulating over time. These compounds may also be metabolised in the environment, potentially de-brominating into more bioavailable congeners. Wilson *et al.* (1997) found that for biosolids applied to soils, some compounds such as PCBs, chlorophenols and volatile organic compounds (VOCs) reached background levels after 128 days, however some compounds persisted (PCDD and PCDF) beyond 260 days of monitoring. Biodegradation and volatilisation were the primary removal mechanism for the compounds that were removed. Compounds such as carbamazepine have also been found to be resistant to degradation and show the potential to build up over time (Gibson *et al*. 2010).

In addition to studies identifying the persistence of some compounds in sludge amended soils, a few have also examined mobility of POPs. Dizer et al (2002) found that applying biosolids to land may cause both soil contamination and transport of endocrine disrupting compounds to surface and/or groundwater as contaminants move from biosolids into the environment. Edwards *et al.* (2010) also studied the movement of compounds from biosolids amended soils into drainage water. The study found that for acetaminophen, naproxen, ibuprofen, gemfibrozil, carbamazepine cotinine, fluoxetine, atenolol, sulfamethoxazole, triclosan, and triclocarban in tile drainage below biosolids amended soil, none of the substances were present at ecotoxicologically relevant concentrations apart from triclocarban. Their study also found that the method of sludge application affected the relative levels of PPCPs in tile drainage. Where dewatered sludge (biosolids) was applied, much lower concentrations of POPs were found in drainage water compared to where liquid sludges had been applied. In a long-term study of sludge amended soils, Gibson *et al.* (2010) found that all the micropollutants studied leached to some extent although endocrine disruptors, which are generally less polar than acidic pharmaceuticals, were less likely to leach due to their low solubility at environmental pH. Acidic pharmaceuticals were found to have increased solubility at higher pH, which may encourage leaching. Gibson *et al.* (2010) found that pollutant concentrations of soil leachates were highest for samples that had been irrigated with liquid municipal wastewater effluent for 40 years compared to those irrigated for 10 years. Unexpectedly, a site irrigated for 90 years was of medium concentration suggesting that potentially the soil microbiome was better adapted to biodegrade some of the pollutants applied, or other physicochemical removal mechanisms such as mineral binding sites had been depleted over time, providing a reduced retention capacity compared to soils with shorter history of irrigation.

The study by Gibson *et al*. (2010) found that organic matter content was the most important feature of soils in determining leaching potential, with higher organic matter resulting in less pollutant leaching. This may explain the results in the study by Edwards *et al.* (2010) comparing dewatered versus liquid sludges. Organic matter content has been found to be important to both POP mobility and inorganic pollutant mobility. Inorganic pollutants such as heavy metals are also found to adsorb to biosolids (Haynes *et al.* 2009). Their relative mobility in the environment has been found to be influenced by sorption to organic matter, pH, nutrient content and soil conductivity (Alloway and Jackson 1991). The release of metals from biosolids has also been found to be greater at the first application of biosolids, during decomposition, when metals bound to organic matter may be leached more readily. Formation of complexes between metals and organic matter contributes to heavy metal mobility (McBride *et al.* 1997). Over time, there is a reduction in mobility, hence understanding fluxes of organic matter before land application may be important to understanding movement of trace metals from biosolids into soils (Haynes 2009). There is also some evidence that treatment processes within the WWTP can affect the association between organic matter and metals. Biosolids from plants with more mineralizable nitrogen content will undergo ammonification and nitrification in soil, a process that is acidifying overall (Hooda and Alloway 1994). This can enhance metal transport. Therefore, nitrifying treatment plants where reduction in mineralisable N, and by association degradable organic carbon, has already taken place may demonstrate reduced metal mobility compared to non-nitrifying plants.

There have been only a few studies that have looked beyond the accumulation and mobility of biosolid contaminants to consider ecological or ecotoxicological effects. The application of biosolids has been found to decrease soil pH and increase soil conductivity, with higher application rates having a more profound effect (Antilen *et al.* 2014). Cation exchange capacity has also been observed to increase however this increase may be delayed 4-6 months after application (Antilen *et al.* 2014). These factors may influence pollutant mobility or bioavailability. Carbonell *et al.* (2009) carried out a soil microcosm study using multi-species soil system to simulate conditions of application to agricultural land and the potential ecotoxicological impacts on agricultural land. The study found that the application of sludge decreased soil pH from a control of 8.84 to 8.08 and increased soil conductivity. The levels of microbial enzymes were found to be higher in sludge amended soils compared to controls, and body weight of earthworms increased compared to the control. The authors suggest that increased body weight could also be associated with increased bioaccumulation of some toxic substances, but the authors admit that the application of sludges to soil produces complex responses and contradictory effects on various pathways and parameters leading to unclear dose responses. Other studies have confirmed that application of biosolids to land can potentially increase soil contamination and transport of harmful substances to surface and/or groundwater, and in some cases lead to bioaccumulation of some toxic substances in plants, and soil organisms, including food crops (Chaney *et al.* 1996; Dizer *et al.* 2002; Carbonell *et al.* 2009; Malchi *et al.* 2014). Land application of biosolids can potentially result in exposure pathways such as inadvertent digestion for grazing animals, providing a pathway of exposure for humans (Wild *et al.* 1994; Wilson *et al.* 1997).

The studies outlined above provide an indication of the diverse fates of many organic pollutants in biosolids once they reach the environment. The studies indicate that properties of the compounds, characteristics of the biosolid matrix (e.g. organic matter content) and environmental factors (e.g. pH) can affect transport and possible uptake by environmental receptors. While these finding are valuable in demonstrating the presence, mobility and possible biological uptake of POPs in biosolids applied to land, they do not provide practical recommendations for assessing relative risk and developing environmental management practices for POPs in biosolids. Instead the diversity of possible fates highlights the need for more universal approaches to assessing risk from organic pollutants in the environment. The following section examines approaches to risks assessment in the context of biosolids derived POPs.

3.4 Assessing environmental risk of biosolids application

Environmental regulators often use a limited body of evidence to justify exclusion of organic pollutants in biosolids from monitoring and regulation. The justification is typically based on the predicted low mobility of highly lipophilic substances measured in biosolids (e.g. PCBs, PAHs, PCDDs) resulting in low risk of direct exposure through groundwater or entry into the food chain, and consequently no risk to human health (Clarke and Smith 2011). However, as the review of the literature has so far demonstrated, POPs exist in biosolids, application of biosolids to land can lead to accumulation of POPs in soils and other environmental receptors, and some compounds in biosolids applied to land are mobile and may reach other environmental compartments. These findings suggest that with an increasing body of evidence to suggest biosolids present a risk of organic contaminant exposure, new approaches may be needed to assist environmental regulators in assessing environmental risk.

3.4.1 Approaches to risk assessment of organic pollutants

The potential risk of various POPs in biosolids to human health and the environment is wide ranging and therefore difficult to predict. Several researchers have proposed ways of prioritizing organic compounds for monitoring. Besse and Garric (2008) suggest shortlisting human pharmaceuticals in surface waters by their exposure risks, availability of eco-toxicological or pharmacological data of mechanisms of action and adverse effects, and physiochemical data to select priority compounds of a similar chemical structure and mechanism of action. Clarke and Smith (2011) use a ranking system for chemical compounds based upon persistence in soil, potential for human health impacts, evidence of bioaccumulation and eco-toxicity. O’Connor (1996) suggested that the number of compounds for consideration could be reduced if risk assessment was based on pollutants that are frequently detected, resistant to degradation, tend to accumulate in the food chain and are known to be toxic to plants and animals, an approach historically used to prioritise organic compounds such as PCBs or organochlorine pesticides for monitoring and regulatory controls in the past. A brief overview of various approaches to POP risk assessment are discussed below.

3.4.1.1 Persistence, Bioaccumulation and Toxicity (PBT)

Shortlisting POPs for monitoring has been carried out in some countries based on the key criteria of persistence, bioaccumulation and toxicity (PBT). This approach also forms the basis of identifying priority chemicals under the Stockholm Convention. However robust, the approach is limited in its practical application. In Canada for examples, an assessment of 11,317 industrial chemicals for the PBT criteria found that 5.5% were classified as potential candidates for monitoring. This amounts to 622 compounds for possible monitoring. Other studies have found that about 1-2% of all industrial chemicals meet the combined PBT risk factors (Muir and Howard 2006). Most regulatory authorities do not have the resource capacity to carry out monitoring for such a large number of potential pollutants.

3.4.1.2 Risk Quotient (RQ)

The risk quotient (RQ) method has been used widely to estimate risk in the context of organic pollutants such as pharmaceuticals in the environment (Al Aukidy *et al.* 2012; Martin *et al.* 2012; Verlicchi and Zambello 2014; Mansour *et al.* 2016). The RQ is calculated on a chemical-by-chemical basis and is a ratio of the measured (or predicted) environmental concentration (PEC) and the predicted no effects concentration (PNEC), or the maximum concentration at which no adverse effects are predicted to occur (Besse and Garric 2008; Al Aukidy *et al.* 2012; Verlicchi and Zambello 2014). The PNEC is calculated using the “no observable effects concentration” (NOEC) for the most sensitive species and may include a safety factor. A RQ > 1 indicates that the environmental concentrations are present at a level that is likely to have an adverse effect on the target. Martin *et al.* (2012) used criteria of low risk (RQ 0.01 to 0.1), medium risk (RQ = 0.1-1) and high (RQ > 1) for assessing pharmaceuticals in municipal wastewater sludge and compost in a single dose to land. The RQ has been widely applied elsewhere for understanding risk in aquatic environments. It relies on toxicological information being available for a substance, along with a reliable prediction or known environmental concentration.

These measurements are often unknown or based upon a limited number of studies or measurements.

3.4.1.3 Systems approach

Other authors have proposed taking a whole system approach considering the PBT criteria for compounds but also other measures such as relative levels of production, disposal, measured environmental concentrations and likelihood of exposure to create more complex models. Mansour *et al.* (2016) examined the range of criteria that have been used in assessment of pollutant risk. These range from direct concentration measurements to literature-based criteria (e.g. metabolic excretion factors, municipal wastewater treatment removal rates, pharmacology, physiochemical properties, mammalian toxicity, eco-toxicity), and criteria developed from combined measures (e.g. PBT, exposure levels, calculated toxicity or measured toxicity). Figure 3-3 summarises criteria that have been used in previous studies to prioritise pollutants.

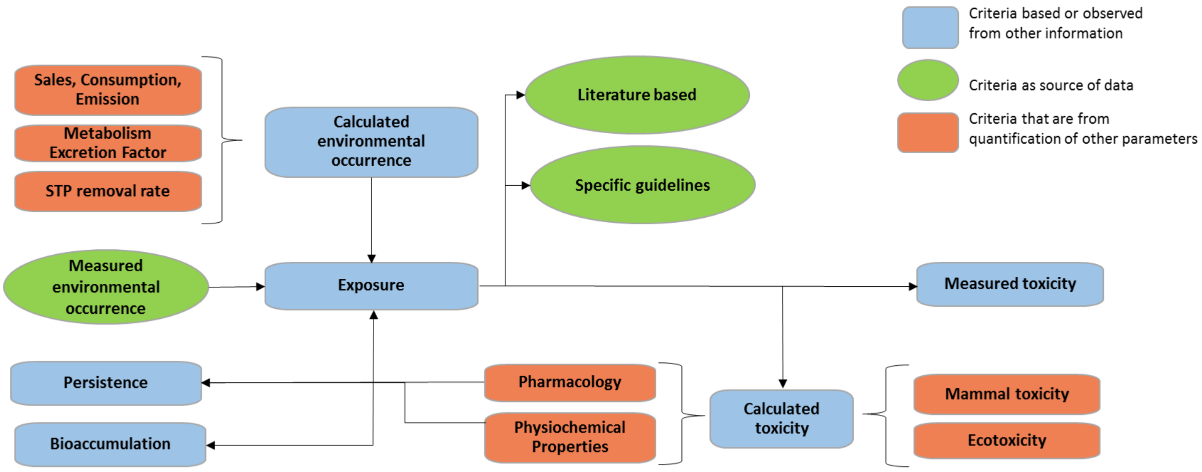


Figure 3-3 Summary of criteria used in previous prioritisation studies (Adapted from Mansour et al. 2016)

Although the approach suggested by Mansour *et al.* (2016) that combines exposure data and PBT screening could potentially reduce the shortlist of compounds identified in PBT screening alone, it is a laborious and time-consuming exercise to calculate, with accuracy, each of these variables. Models would also change as changes in chemical usage occur, updated toxicological data becomes available, or new chemicals come into use. In a similar approach, Besse and Garric (2008) used PEC alongside ecotoxicology, pharmacological data (mechanisms of action and adverse effect) and physicochemical data to estimate a predicted level of harm and to prioritise the most harmful compounds based on potential to cause harm at the PEC, followed by prioritisation of other compounds with similar chemical structures and mechanisms of action. The methods suggested by Mansour *et al.* (2016) and Besse and Garric (2008) provide an indication of how approaches to risk assessment are becoming more refined over time as more data becomes available for assessment of individual compounds. This also provides an indication of the increasing level of complexity required for comprehensive risk assessment on a compound by compound basis. In addition, as many of the criteria used in the approaches have several built-in assumptions, there are also likely to be high levels of uncertainty in the results. As complexity and uncertainty increases, the likelihood of these types of approaches being adopted by environmental regulators will decrease.

3.4.1.4 Approaches for compound mixtures

The approaches listed above are limited to compound-by-compound assessment and do not consider effects of compound mixtures, which may include synergistic effects, which are not easily quantified. Among ten recommendations made by Ågerstrand *et al.* (2015) for improving environmental risk assessment for medicinal products in the environment, include performing mixed toxicity assessments to check for additive effects of some compounds. Some risk assessment approaches for assessing the effects of compound mixtures have been developed. Techniques include concentration addition (CA) and response addition/independent action (IA). Both require qualitative and quantitative knowledge of the mixture and assume the same experimental endpoint for the PNEC. CA assumes that each compound acts in a similar manner and contributes a percentage to the overall toxic effect whereas IA assumes the overall effect of a mixture is a sum of the individual effects (scaled from 0-1) in the substance. CA may be appropriate for compounds with similar molecular mechanisms of action, but for compounds that have different mechanisms of action, IA may be a better predictor of effect. These approaches may be difficult to apply when compound mixtures contain hundreds or even thousands of parent compounds, metabolites and unknown substances. The toxicity prediction is based on known or measured compounds but does not take account of emerging pollutants or unknown breakdown products or metabolites and does not account for synergistic or antagonistic effects. The effects of pH, organic matter content and other environmental factors may also present some uncertainty in the calculated toxicities. CA and IA also exclude considerations of natural behaviours such as feeding rate or activity of organisms (Backhaus *et al.* 2003; Ågerstrand, *et al.* 2015).

3.4.1.5 Limitations of current risk assessment approaches

Four key limitations emerge from the PBT, RQ, systems models and use of compound mixture approaches described above. First, while the global library of published data on eco-toxicological effects for various compounds is increasing, PNECs may currently not be available for a large number of compounds, particularly for new or emerging compounds. In addition, where eco-toxicological data does exist, it may be only for a single, or limited number of target organisms, and may not be considered under different environmental conditions. EU eco-toxicity assessment have typically been based on simplistic bioassays using a set of organisms such as bacteria, algae, daphnids and fish (Backhaus *et al.* 2003; Geissen *et al.* 2015). Second it is difficult to determine the relevance of some of the simplistic toxicity measures (where they exist) across various trophic levels and time scales. Typically, the risk assessment approaches listed do not take account of pollutant pathways in the environment and longer term ecological impacts and chronic effects. Third, these tests cannot account for environmental effects such as physicochemical interactions in the mixture, the availability of compounds for uptake by organisms, and relative physiological responses to mixtures (Backhaus *et al.* 2003). Environmental factors may affect relative toxicity, which is typically unaccounted for in laboratory performed toxicity tests. For example, the influence of factors such as environmental pH and DOC have been shown to affect toxicity by affecting bioavailability or ease of uptake for some compounds (Katsoyiannis and Samara 2007; Neale *et al.* 2011; Rowett *et al.* 2016). A better understanding of the movement of pollutants from biosolids into the environment and their relative bioavailability may be as important as improved understanding of absolute concentrations or individual eco-toxicological effects measured in a laboratory (Clouzot *et al.* 2012). Finally, as new complex approaches attempt to take account of some of the limitations of existing risk assessment techniques, the practicality of application begins to reduce as complexity increases.

3.5 Conclusion

Land application of biosolids is increasingly being used as a primary disposal method for municipal wastewater treatment sludges. The literature review has found that the environmental impacts of this practice, particularly related to organic pollutant transfer into the environment, have not been widely studied, and environmental management approaches are far from robust. The literature does however provide evidence of the occurrence of POPs in biosolids. Hundreds of organic compounds, including PPCPs, have been detected at appreciable quantities in municipal wastewater sludges and biosolids. There is also evidence in the literature that POPs are detected in the soil and groundwater beneath sludge amended soils suggesting pollutant transfer as well as persistence of sludge derived pollutants in the environment. These findings suggest that greater effort to assess the environmental risks of land application of biosolids is needed. Current approaches are limited in their applicability due to the need for extensive analytical effort in the detection and quantification of a large number of chemical compounds. Application of effective risk assessment practices using ecotoxicological data is limited by lack of data and complexity of determining RQs when there is little or no knowledge of predicted environmental concentrations or additive effects of compound mixtures. The current risk assessment approaches are time consuming, costly and carry large levels of uncertainty and may not be practical for use by environmental regulators around the world. Thus, there is a need for alternative techniques that can allow for an assessment of relative risk based on methods that could be more widely applied and could be used for both routine characterisation of biosolids. Such approaches could also enhance the comparison of treatment plant stages or treatment parameters to refine removal mechanisms for POPs in biosolids. A proposed alternative that would overcome some of the key barriers currently faced could include:

- relatively simple and accessible analytical methods;
- ability to account for the vast numbers of potential pollutants without a need to identify individual compounds, transformation products and other unknown organic pollutants;
- ability to estimate exposure potential (e.g. pollutant mobility) in contrast to absolute concentrations;

- a bulk quantification that can be used as an indicator of treatment effectiveness.

The following chapter will explore components of various assessment tools that could be used to construct an approach based on the properties listed above.

CHAPTER 4 LITERATURE REVIEW: ALTERNATIVE APPROACHES TO ASSESSING RISK OF POPS IN THE ENVIRONMENT FROM LAND DISPOSAL OF BIOSOLIDS

4.1 Introduction

The literature has described the lack of application of practical regulatory controls for POPs in biosolids in most countries around the world, even though there is evidence that POPs are present in biosolids and these substances can be mobile and persistent in the environment. The literature also highlights the limitations of existing approaches to risk assessments for POPs. There is a clear need for alternative strategies to be considered. This section examines approaches to determining key characteristics of biosolids that could be used to quantify environmental risks. This includes assessment of biosolid-derived POP mobility, bulk quantification measures for organic compounds, and assessment of the persistence and composition of the bulk mobile fraction.

4.2 Leaching potential

Leaching procedures have been used by environmental regulators for many years to understand the movement of pollutants from materials such as reclaimed soils or solid waste into the environment. Principles of solid waste leaching procedures include the use of a solvent (typically water or a weak acid) that is mixed or shaken with the solid material under fixed conditions of time, temperature and mixing speed to simulate natural leaching processes (Morissette *et al.* 2015; Tiwari *et al.* 2015). These procedures are intended to replicate accelerated environmental conditions to estimate the degree to which adsorptive bonds between pollutants and solid particles may be broken. This is then used to provide an estimation of how pollutants may transfer between the solid matrix and the aqueous environment under conditions such as rainfall in the environment. Municipal wastewater sludge is a complex mixture that can have appreciable concentrations of multiple organic compounds sorbed to solid particles by mechanisms including hydrophobic bonding, ion exchange complexation or hydrogen bonding as described in Chapter 2 (da Silva *et al.* 2011). Desorption from solids is an important factor in understanding relative exposure rates and potential for degradation of organic pollutants in the environment through volatilisation, chemical or microbial degradation (Wang and Jones 1994). Chemicals demonstrate various levels of association with solids in wastewater effluent (Carballa *et al.* 2004; Barret *et al.* 2010; Baker *et al.* 2012) and not all compounds follow a simple first order desorption model from biosolids into the environment (Purdy and Cheplick 2014), however leaching tests can provide an estimation of mobile compounds.

A number of methods have been developed over the decades to assess the risk of pollutant leaching potential from waste materials, such as the toxicity characteristic leaching procedure (TCLP), solid waste extraction procedure (SWEP), US Environmental Protection Agency (USEPA) methods and desorption experiments performed by others (e.g. Wang and Jones 1994). Currently there are a limited number of tests that have been validated for use in measuring the leachability of non-volatile organic compounds from solid wastes and soils, with most tests designed for simulation of leaching of inorganic compounds (e.g. heavy metals). Table 4-1provides a comparison of the leaching tests most commonly used by environmental regulators.

Table 4-1 Comparison of leaching tests potentially suitable for assessing the leaching of POPs from biosolids.

Test	Test conditions	Applicable test matrices	Extraction fluid	Duration	Liquid to solid ratio (m:m)	Shaking speed	Reference
Synthetic Precipitation Leaching Procedure (SPLP) SW 846 Method 1312	Aerobic condition, leaching from material exposed to surface rainfall;	Organic and inorganic pollutants in soils and biosolids	nitric/sulphuric acid, pH 4.2	18 h	20	30 rpm	[1, 3, 4]
Toxicity Characteristic Leaching Procedure (TCLP)	Aerobic condition, leaching from landfilled material; shaking test	Organic and inorganic pollutants in soils and biosolids	acetic acid, pH 4.93	18 h	20	30 rpm	[1, 2, 3]
Dutch Total Availability Leaching Test (NEN 7341)	Aerobic conditions; two pH conditions	Typically, only for inorganic constituents; matrix must be ground to a fine powder	1 M nitric acid, pH 7 and 4	6 h (2 steps)	50	n/a	[1, 3]
EN 12457-2 (EU)	Aerobic conditions, Simulates exposure to rainfall	Typically, only used for inorganic pollutants in granular wastes, mining slag and sludges	DI water, pH 5.75	24 h	20	10 rpm	[3]
Standard Test Method for Leaching Solid Material in a Column Apparatus ASTM D 4874	Aerobic conditions, column test; upflow through column; 5000 g, max particle size 10 mm	Low concentration organic and inorganic pollutants in low aqueous solubility media	DI water	24 h	n/a	n/a	[1]
Dutch Column Test (NEN 7343)	Aerobic conditions, column test; particle size <4 mm	Inorganic pollutants in soils and stones	DI water, pH 4	21 d (7 extractions)	0.1-10	n/a	[1]
Sequential Batch Leachate Test (SBLT)	Anaerobic conditions, landfill leachate;	Non-volatile organics and inorganics from landfill leachate	DI water	24 h x 4 (4 batches)	4	40 rpm	[1]
Solid Waste Extraction Procedure (SWEP)	Aerobic conditions, shaking test	Inorganic pollutants from metallurgical and mineral wastes	acetic acid, pH 5	24 h	20	10 rpm	[1, 3]

[1] Washington State 2003; [2] Tiwari *et al.* 2015; [3] Rene *et al.* 2017; [4] USEPA 2017

Of the tests reviewed, those that have been validated for use to assess the leachability of non-volatile organic constituents included column tests such as ASTM D 4874, and shaking tests such as the sequential batch leachate test (SBLT), TCLP and the USEPA synthetic precipitation leaching procedure (SPLP) SW 846 Method 1312 (Washington State 2003; USEPA 2017). The column tests (e.g. ASTM D 4874) were found to be less suitable for samples that have high aqueous solubility, such as municipal wastewater sludges. The shaking tests were found to provide a more suitable option for this matrix, however the SBLT test is designed for assessing leaching under anaerobic conditions (e.g. landfill leachate). Biosolid application to land can include both surface deposition, and injection into soils, however conditions are not expected to match those anaerobic conditions found in a landfill and will most closely match aerobic conditions, therefore the SBLT is unlikely to be suitable for simulating organic pollutant leaching from biosolids. The TCLP test is a widely used test for contaminated land studies, and many laboratories have accredited methods for carrying out TCLP testing for reclaimed soils, mine spoils, and other solid waste materials. The leaching fluid used in the TCLP test however is acetic acid (CH_3COOH). If considering bulk quantification measures such as total or dissolved organic carbon (DOC) of the organic leachate from a test such as the TCLP, the presence of acetic acid would provide a significant interference and potential source of error. The SPLP tests mirrors the conditions of the TCLP test closely, with the key difference being that the leaching fluid does not contain organic interferences (dilute nitric/sulphuric acid: HNO_3/H_2SO_4). The SPLP tests could be a suitable alternative to the TCLP test for evaluation of organic compound leachability from biosolids.

4.3 Alternative quantitative technique for POPs in biosolids

The literature demonstrates that chemical-by-chemical analysis provides a time consuming and resource intensive process. Over time, it is likely that priority organic compounds will change and subsequently new methods for analysis and setting regulatory limits will be required. As many organic compounds are phased out of use (e.g. PCBs), environmental regulators will be left with analytical and regulatory protocols that are no longer fit for purpose, requiring new analytical baseline measurements and analytical tools to be developed for emerging contaminants (Harrison *et al.* 2006).

The use of indicator compounds as a benchmark for relative pollutant burden has been proposed to overcome some of the challenges associated with assessing a large number of individual substances (Barber *et al.* 2005; Anumol *et al.* 2015). Features of good indicator compounds include ease of analysis, widely present at detectable levels, and as persistent as the most persistent compounds of interest (Anumol *et al.* 2015). However, identifying suitable indicators is challenging given that not all classes of organic pollutants or compound types behave similarly under all conditions encountered in the wastewater treatment process or in the environment as examined in Chapter 3 (Gros *et al.* 2010). Carbamazepine for example has been proposed as a good indicator due to its high persistence in the environment and origin almost exclusively from wastewater treatment systems (Mompelat *et al.* 2009; Gasser *et al.* 2011). However, the presence of carbamazepine may only provide an indicator that pollution from municipal wastewater is present, with no quantification of risk. Another limitation is that the presence of carbamazepine is typically associated with use of epilepsy drugs. The presence of the compound may vary depending on the relative level of prescribing in a given WWTP catchment area, and if hospital wastewater makes up a proportion of the source. In addition, while this compound could be used as an indicator of effluent pollution, it is known for its lack of partitioning to biosolids and is unlikely to provide a good indicator of pollution originating from a biosolid material. Similar difficulties will be encountered for most candidate indicator pollutants (e.g. uncertainty due to the relative level of use in the given catchment, variation in partitioning behaviour etc.) suggesting that a more suitable approach is to select compounds that represent a characteristic common to the pollutants of interest, such as environmental persistence or non-biodegradability (Clouzot *et al.* 2012). This could potentially include more ubiquitous measures such as persistent or recalcitrant fractions of total organic carbon (TOC).

TOC has long been considered a useful parameter for estimating organic pollutant loading into the environment, and wastewater treatment performance (Aziz and Tebbutt 1980; Fadini *et al.* 2004; Dubber and Gray 2010). TOC provides a comprehensive measure of oxidisable organic matter (OM) including both readily biodegradable and recalcitrant OM. TOC measurements are used widely in the pharmaceutical manufacturing industry to detect residues of pharmaceutical products (antibiotics, steroids, antinauseants and biopharmaceuticals) in production systems, as a way of determining the cleanliness of the production process (Jenkins *et al.* 1996; Clark, 2000). The non-specific methodology saves both time and money by providing a quick, generic test for organic compounds. Others have successfully used TOC analysis to screen for petroleum hydrocarbon pollution on industrial sites, and as an indicator for ground and surface water contamination near refineries, and an indicator of persistent organic pollutants in soils (Spruill 1988; Schreier *et al.* 1999; Nam *et al.* 2008). In a study of contaminated soils by Nam *et al.* (2008), a very significant correlation between TOC and hexachlorobenzene (HCB), PCBs, PBDEs ($p < 0.001$), and significant correlation for PCDD/Fs ($p<0.05$) was found across the soils studied.

The literature indicates that TOC is a good generic indicator of organic pollutant burden and can be assessed using relatively simple and inexpensive analytical methods. Analysing the dissolved fraction (following filtration using 0.45 μm pore size filters) allows for interferences such as bacterial cells to be excluded from analysis and has been shown in the literature to be more representative of organic pollutant fraction as compared to bulk organic matter content. Aziz and Tebbutt (1980) found that the ratio of biological oxygen demand (BOD) to TOC was always higher in unfiltered municipal wastewater as compared to filtered, suggesting that a greater proportion of the filtered fraction is non-biodegradable. There is evidence that DOC may also be related to relative toxicity. Van Loon *et al.* (1997) found a surprising correlation between estimates of total body residue (TBR) in organisms exposed to treated effluent and measured DOC concentrations in these effluents, with the strongest correlations found for organochlorine and organobromine compounds. This correlation was not observed for surface waters, where it is predicted that the high concentrations of humic substances and natural organic matter (NOM) will be responsible for most of the DOC in surface waters but was suitable for municipal wastewater where the DOC is more likely to represent non-humic or non-NOM sources of organic carbon. DOC leached from biosolids may behave as a carrier for hydrophobic pollutants increasing the water solubility of lipophilic organic pollutants and facilitating transport in the environment (Hasset and Anderson 1982). Katsoyiannis and Samara (2007) found a good negative linear relationship between DOC in WWTP and the log K_d (desorption coefficient) of persistent organic pollutants. They found that DOC increased the water solubility of organic pollutants, facilitating transport through the treatment system and influencing the distribution of POPs in wastewater treatment compartments. Increased DOC concentrations caused a decrease in the ratio of sorbed to dissolved POPs, and thus acted as a microscopic pseudo solvent phase carrying POPs in treated effluent (Katsoyiannis and Samara 2007). The potential transfer of compounds into the environment may therefore be influenced by the relative quantity of DOC that is desorbed from solids and transfers into the environment under leaching conditions. Measurements of leachable DOC may be an important parameter in understanding the potential for POP pollution from land application of biosolids, and quantifying DOC fluxes from biosolids may assist in understanding POP transport in the environment more broadly.

4.4 Alternative qualitative analysis

4.4.1 Biodegradability

The above section proposes that leachable DOC from biosolids may provide a useful indicator of organic contaminant fluxes into the environment. However, further processing and analysis of this fraction could assist in the characterisation of the DOC, particularly the relative quantity of persistent organic carbon that is present. As described in the review of PBT approaches earlier, the persistence of a compound is a key indicator of its potential to be included for environmental monitoring. Risk assessment often applies degradation models that estimate the rate of decline of compounds in the environment after discharge or application and a key characteristic of many of the most harmful organic pollutants in the environment, is their persistence and resistance to degradation in treatment processes.

The rate of decline in organic substances such as pesticide concentrations are often approximated as simple exponential decay, equivalent to a first-order degradation model of chemical reactions (Purdy and Cheplick 2014). However, not all compounds follow simple first order decay models, and for chemical mixtures, the rate of decay is difficult to estimate. As previously highlighted in Chapter 3, Torri *et al.* (2003) found that in biosolid amended soils, two key fractions of organic carbon were identified by their biodegradability; the quickly mineralised fraction (53-71%)

and a resistant fraction (29-45%). The relative biodegradability is affected by differences in chemical structures, but also by the microbial community and the physical conditions present (e.g. temperature) (Wang and Jones 1994; Onesios *et al.* 2009).

Existing test methods for ready biodegradability include the Organisation for Economic Co-operation and Development OECD Guideline for Testing of Chemicals 301 (17.07.92), which is used for individual chemicals over 28 days. Other methods include those that estimate biodegradable dissolved organic carbon (BDOC) using the difference between initial DOC and the minimum reached during the incubation period of up to 28 days (Trulleyová and Rulík 2004; Knapik *et al.* 2015). The BDOC procedure is suitable for assessing the potential degradability under aerobic conditions and for most compounds aerobic degradation is the primary biodegradation pathway (Barret *et al.* 2012). Some compounds are biodegradable under both aerobic and anaerobic conditions (e.g. naproxen) (Carballa *et al.* 2007b) whereas others show limited degradation in aerobic conditions (Barret *et al.* 2012). For some resistant compounds, particularly longer chain PCBs, a more significant degradative pathway may occur in an anaerobic environment where hydrogen sulphide is present, by the process of reductive de-chlorination (Rogers 1996). For example, sulfamethoxazole, which has been found to be resistant to aerobic biodegradation, shows a high removal efficiency (> 80%) under anaerobic as compared to aerobic conditions (Carballa *et al.* 2007a). These principles could be applied to define a biodegradation approach that allows for persistent desorbable dissolved organic carbon (PDDOC) to be quantified, under both aerobic and anaerobic conditions. This could be used to inform the additional sludge processing treatments that could be undertaken to reduce organic pollutant burden in biosolids.

4.4.2 Leachate characterisation

In addition to the approach listed above, characterisation of leachates could be carried out that goes beyond determination of the persistence of bulk DOC in leachates. Further detailed analysis using sophisticated analytical equipment (e.g. LC-MS/MS) could be used to characterise the remaining compounds. A disadvantage of using detailed analytical approaches relates to the previous challenges associated with selection of indicator compounds, or requirement to measure a large number of individual compounds. Other techniques that provide a broader characterisation such as ultraviolet spectroscopy (UV-Vis) wavelength scans or Fourier transform infrared (FTIR) analysis could be applied instead. These techniques are inexpensive and relatively easy to use and can be used to identify general characteristics that could indicate the presence of certain pollutant types, or to compare leachates from different source material or treatment stages.

The use of UV-Vis is common in the study of dissolved organic matter (DOM), and may be useful in application to other disciplines such as biosolid leachate characterisation (De Haan and De Boer 1987; Weishaar *et al.* 2003; Helms *et al.* 2008; Krasner *et al.* 2009; Knapik *et al.* 2015). UV-Vis is often used to measure changes in the dominant DOC peak of 254 nm in experiments evaluating the biodegradation of anthropogenic (wastewater derived) organic matter in river waters. Li *et al.* (2015) used UV-Vis spectra to characterise changes in dissolved organic matter during municipal waste composting. A review by Li and Hur (2017) highlights the ease of use of the technique and notes applications for measurement of both DOC and trace organic pollutants. The technique can go beyond using single wavelengths to the use of wavelength scans that allow for absorbance ratios to be calculated that can infer molecular weight, aromaticity and the sources of DOM.

FTIR has been used to examine DOM leachates in a range of applications, identifying the main functional groups, and characterising differences between treatment methods (Soong *et al.* 2014). Provenzano *et al.* (2011) demonstrated that FTIR was useful in characterising final digestate products obtained from different source materials. Reinhart and Bolyard (2015) used FTIR to identify various aromatic, organic, inorganic, and nitrogen functional groups in landfill leachates over time to determine when a landfill had stabilised. A change in the dominant functional groups demonstrated a shift from leaching of organic to inorganic substances.

Mesquita *et al.* (2017) provide a comprehensive review of where multiple spectral techniques, including UV-Vis and FTIR can be used individually or in combination with each other in the characterisation of wastewater effluents and other complex matrices to understand effects of processes, comparison of treated and untreated samples, and to measure changes over time. The review highlights the benefits of these techniques due to their low cost, and reduced requirements for sample processing and handling compared to complex solvent extraction techniques and sophisticated analytical approaches. The combination of techniques such as UV-Vis and FTIR could thus be useful for both examination of dominant functional groups in leachates, but also to compare leachates following different treatments or derived from different source material. For biosolid leachates, this could allow for greater understanding of the effects of wastewater treatment processes on the quantity and type of persistent dissolved organic carbon found in leachate.

There are some limitations, however. For example, in UV-Vis analysis, interference from suspended particles can affect accuracy of hydrocarbon measurements due to light scattering, however, use of filtered samples will help to overcome this barrier (Li and Hur 2017; Mesquita *et al.* 2017). Bands may also be broad and non-specific limiting the precise identification that could be obtained using other more sophisticated techniques like LC-MS. Similar limitations exist for FTIR analysis, and interferences from water in the sample can mask some of the precision of higher transmittance values. FTIR, which has typically been used for single compound analysis, is limited in mixtures by identifying only the dominant functional groups therefore lacking some precision. Accurate identification of individual compounds is not possible; however, identification of certain functional groups can provide an indication of the types of compounds that may be present.

4.5 Conclusions

The previous chapter identified the need to identify new approaches to assist in assessment of relative risk of organic pollutant transfer from biosolids into the environment. This chapter has provided an overview of the key components of a suitable approach, which include:

- Experimental procedures that improve the understanding of pathways of exposure, such as movement of organic pollutants from solid to aqueous compartments, e.g. leaching tests;
- Analytical measurements that bypass the need to measure and monitor all possible compounds and can better estimate the total pollutant burden, where mixtures of compounds, metabolites and transformation by-products exist (non-targeted qualitative screening), e.g. DOC analysis;

- Experimental procedures that characterise the total organic component within biosolids (e.g. biodegradability) derived from various treatment systems to inform wastewater treatment processes to better manage organic pollutants, e.g. biodegradability tests coupled with UV-Vis/FTIR.

The combination of the quantitative and qualitative techniques described above provide components for building a framework for a new approach to assessing environmental risk from the transfer of organic compounds from land application of biosolids. This approach overcomes some of the key limitations of existing approaches. Examples of applying this combination of techniques has not been found in the literature, therefore experimental work applying this approach will contribute to expanding knowledge of biosolids and environmental risk assessment.

CHAPTER 5 SUMMARY OF THE LITERATURE

This section summarises the key findings and themes identified in the literature review. It highlights the key properties of wastewater treatment and composition of biosolids along with considerations of the principle knowledge and practice gaps that provide a justification for the research

Organic pollutants in the environment are an increasingly complex challenge for environmental regulators and government agencies to address. These complexities are associated with the large number of compounds and their metabolites entering the environment and the continual problem of which pollutants to monitor and regulate as the number and diversity of potential pollutants increases. International agreements and global efforts, such as the Stockholm Convention, have prioritised POPs, those which will reach the environment and not be degraded by physicochemical or biological mechanisms based on the legacy of harmful organic pollutants in the environment such as pesticides. With greater awareness of emerging pollutants arising from PPCPs and industrial processes, new efforts are focussing on their principal pathway into the environment, namely wastewater treatment systems and by-products.

5.1 WWTP and POPs

WWTP are not designed for POP removal, and hence there is limited incentive for treatment plant operators to investigate optimisation processes for their removal. Domestic wastewater, hospital effluent and industrial effluents may all be contributing to the diverse mixture of emerging organic pollutants now directed to centralised wastewater treatment facilities. Many authors have reported on detection of emerging POPs in wastewater effluents and increasing evidence of the presence of POPs in wastewater sledges has also been reported. Little attention however has been paid to the effect of wastewater treatment processes on reducing concentrations of organic pollutants in the sludges and biosolids arising from municipal wastewater treatment plants. Principles of wastewater treatment suggest that some organic pollutants will partition to sludges but the sorption process for the diverse collection of POPs is complex and affected by individual chemical properties (e.g. lipophilicity, structure), pH, presence of DOC, and possibly the operational parameters of the treatment process. Sludges from various stages of the wastewater treatment process are likely to contain diverse collections of organic substances but there has been limited study to assess the effect of treatment systems on the proportion of POPs that partition into sludges. Features of WWTP such as operational parameters or additional sludge treatment may affect the quantity and type of pollutants that may be present. Given the potential for WWTP to be major source of the next generation of emerging pollutants, greater understanding of the effect of treatment systems on POPs in biosolids is needed.

5.2 Biosolids and the environment

The practice of land application of the solid by-products of wastewater treatment processes, or biosolids, is increasing around the globe and has provided an alternative to the practice of sludge disposal at sea. It also provides an alternative to the costly practice of landfilling. With vast quantities of biosolids being produced each year, land application is largely becoming a necessary outlet for WWTP operators. In many countries, the practice is encouraged, with the emphasis on the many positive environmental benefits such as returning valuable organic matter to depleted soils, and recycling phosphorus and other nutrients to soil in place of commercial fertilisers. There is clear evidence in the literature however that biosolids are sinks for many organic pollutants at appreciable quantities and these include a wide range of pollutant types. Globally there is very limited regulatory control of these substances, with many authorities using assumptions of no risk, or absence of evidence as evidence of no risk.

Alongside evidence of the presence of organic pollutants, there is also some indication of pollutant behaviour in biosolids amended soils, with pollutants either persisting in environment for many years, or moving into other environmental compartments such as drainage or groundwater systems. The associated risks of organic pollutant transfer from biosolids to the environment remain largely unquantified. There are several reasons for this. The vast number of potential compounds present makes the selection of priority chemicals for monitoring and management a difficult process. In addition, current techniques for preparation and analysis of many emerging pollutants have not been developed, are complex and expensive, or carry high levels of uncertainty due to the complex sludge matrix. Detection capabilities are also limited in most cases to laboratories with expensive and specialised equipment that requires highly skilled operators. These features make routine monitoring of specific organic compounds unlikely.

Analytical limitations aside, existing risk management tools based on toxicology data are limited by the existing library of eco-toxicological studies, often carried out on a single species, or limited to acute testing only, with no estimates of chronic exposure via various exposure pathways and under various environmental conditions. Factors such as pH and the presences of DOC may be important to how pollutants move in the environment. As more refined models and systems level approaches are developed to account for some of these factors, the practicality of applying risk management approaches decreases. The review of the literature has identified both knowledge and practice gaps for the management of POPs in biosolids spread to land. These are summarised in Table 5-1.

Table 5-1 Knowledge and practice gaps for management of POPs in biosolids applied to land

Knowledge Gaps	References
• Lack of toxicity and ecotoxicity data for many compounds; • Lack of knowledge of fate of specific compounds under varying environmental conditions; • Lack of knowledge of chronic effects and population effects; • Limited understanding of the effect of WWTP processes on POPs in municipal wastewater sludges; • Few studies on compound mixtures including metabolites, which could be more toxic than parent compounds; • Lack of evidence of POP transfer from biosolids into the environment; • Lack of comparison of effects of biosolids treatment on POPs; • Lack of understanding bioavailability and environmental degradation potential for many POPs.	Diamond *et al.* (2011); Ågerstrand *et al.* (2015); Gavrilescu *et al.* (2015); McCarthy *et al.* (2015) ; Petrie *et al.* (2015); B.C. Ministry of Environment (2016).
Practice Gaps	**References**
• Lack of methods for quantifying emerging and unknown compounds and compound mixtures, including bulk quantification of compound mixtures; • Lack of regulatory guidelines specifying which compounds to measure, and under what circumstances; • Lack of regulatory guidelines on appropriate environmental limits for monitoring before or following biosolids application; • Lack of regulatory guidance on the effectiveness of treatment technologies for reduction of POP burden in biosolids	Diamond *et al.* (2011); Clouzot *et al.* (2012); McCarthy *et al.* (2015); BC Ministry of Environment (2016) NCR (2002); EC (2018).

5.3 Designing new approaches

The knowledge and practice gaps summarised in Table 5-1 emphasise the need for new approaches for the assessment of organic pollutant risk from land application of biosolids. To better inform future regulatory policy, research approaches must be designed to consider the applicability to practical solutions to minimise risk (Ducey *et al.* 2010; Boxall *et al.* 2012; Clouzot *et al.* 2012; Petrie *et al.* 2015). Tools that can improve understanding of pollutant mobility, provide a bulk measure of organic pollutant burden, and can be used to compare different treatment processes or stages could help address some of the key knowledge and practice gaps that exist. The literature review has identified that leaching tests can assist in predicting mobility and fluxes of pollutants into the environment. The literature also finds that DOC is a good indicator of organic pollution. The qualitative analysis of DOC for biodegradability (PDDOC) and characterisation of leachates by techniques such as UV-Vis wavelength scanning, and FTIR analysis can help to understand and compare biosolids from different treatment plants or stages of a treatment process to evaluate relative pollutant burden.

The following chapters present experimental work designed to characterise PDDOC from various sludges and biosolids obtained from a selection of wastewater treatment systems, and to compare sludge and biosolid leachates from various WWTP types and stages of treatment. The work presented includes the assessment of DDOC from various biosolids, assessment of persistent fraction of the DDOC (PDDOC) and application of qualitative analysis of the biosolids and leachates to before and after biodegradation experiments.

CHAPTER 6 INTRODUCTION TO THE EXPERIMENTAL DESIGN

This section will set out the general methods used in the research. This will include a description of the experimental design, site selection, sample collection, preparation and processing and analytical methods. The approaches to quality control and areas of uncertainty are also presented.

6.1 Experimental design

The experimental work undertaken in this study seeks to generate data that allows for comparison of leachable dissolved organic carbon (DOC) from municipal wastewater sludges and biosolids produced at various stages of the wastewater treatment process. The design is based on the literature discussed in Chapter 4 and will be applied to quantify leachable DOC from wastewater sludges produced at primary and secondary treatment stages, and biosolids after further treatment (e.g. dewatering, thermal treatment or anaerobic digestion), and compare leachates from different types of treatment plants. The leachates will be examined further for relative biodegradability and spectral properties using UV-Vis and FTIR. The experiments set out in the chapters that follow will provide the quantitative and qualitative data required to characterise mobility of persistent organic carbon from sludges and biosolids and compare sludges from different sources and stages of treatment. A diagram of the general experimental approach is shown in Figure 6-1.

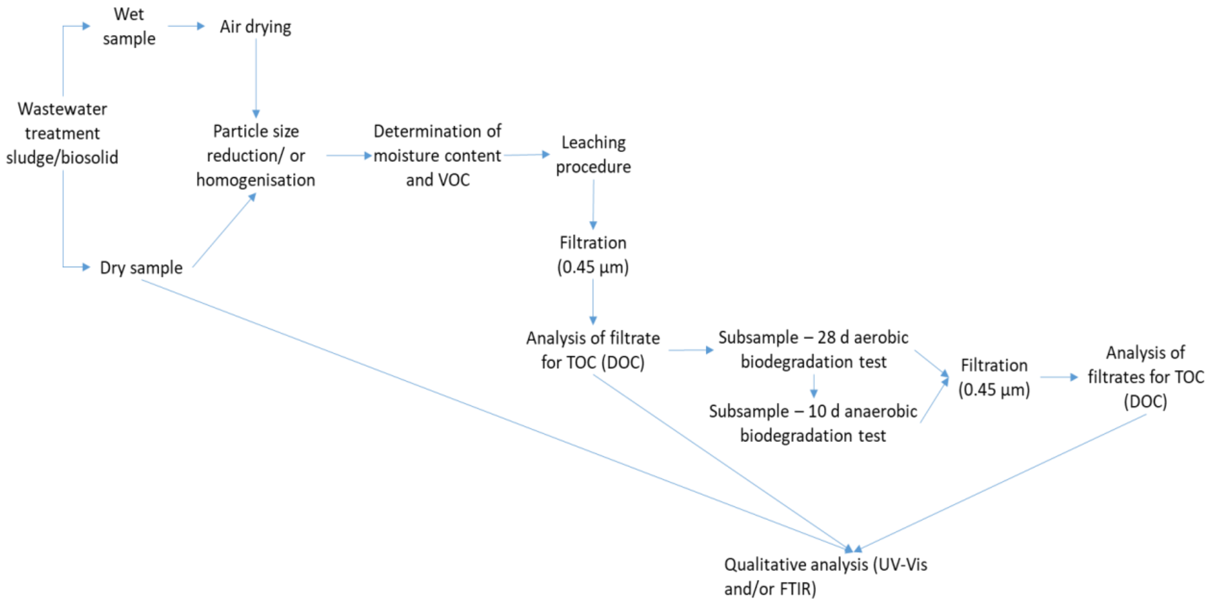


Figure 6-1 Flow diagram of the experimental work

The general methods and materials used for sample collection, preparation and pre-treatment are presented in this chapter. In addition, the analytical methods used for determination of DOC are presented. DOC is used as a general parameter throughout the experimental stages of this study to quantify the leachability of organic compounds, or DDOC and the persistence of DDOC (PDDOC). Detailed methods specific to the desorption experiment; the biodegradation experiment; and qualitative analysis of leachates are presented separately in their respective chapters (7-9).

6.2 Site selection

Four WWTP of various sizes and treatment types were identified as sources for sludge and biosolid collection in this study. The sites were chosen to include plants where sludges could be obtained from various treatment stages (primary settled sludge, secondary sludge, and a final biosolid or sludge product). In addition, plants with varying levels of nitrification were included (nitrifying, partially-nitrifying and non-nitrifying processes). Samples were collected in the spring between April and June, when temperature is more representative of annual averages. This spring period also avoided potential seasonal loading of pharmaceuticals due to spikes in occurrence of winter cold and flu, or changes in loading associated with summer absences in schools and universities and increases in tourism. Table 6-1 lists the treatment plants that were used in this study.

Table 6-1 Sample locations selected for sludge collection in this study.

Plant ID	Plant Size			Treatment Stage			Treatment Type	
	Small	Medium	Large	Primary	Secondary	Sludge cake or pellets	Nitrifying system	Non-nitrifying system
WWTP_1			237,000 pe	✓	✓	✓		✓ (HRT 4 h)
WWTP_2	2,893 pe			✓	✓		✓ Primary settlement + oxidation ditch (HRT 20 h, SRT ~ 25 d)	
WWTP_3		21,364 pe			✓	✓	✓ Oxidation ditch plus secondary aeration (HRT 18-27 h, SRT ~6d)	
WWTP_4		21,800 pe			✓	✓	partially nitrifying . CASS system SBR, (HRT 4+ h)	
Reference Material	CRM 055: Domestic sewage sludge, after final processing and thermal stabilisation							

pe = population equivalent

6.2.1 Description of the WWTP

WWTP_1: A large municipal wastewater treatment plant receiving wastewater from storm drainage, domestic, commercial and industrial premises including pharmaceutical manufacturing as well as a large hospital, and two universities. The treatment stages undertaken at the plant include grit removal (6 mm screen), primary settlement, secondary aeration followed by settlement, and discharge of treated effluent to an estuarine environment. Sludge treatment includes anaerobic digestion (AD) (mesophilic process, 35°C), pH adjustment, dewatering (belt press) and thermal drying at 120°C to create a sludge pellet biosolid that is sold as fertiliser.

WWTP_2: A small municipal wastewater treatment plant receiving storm water and wastewater primarily from domestic premises and a small number of commercial premises (e.g. restaurants, hotel, shops). Treatment is relatively basic compared to WWTP_1 and consists of a primary settlement tank followed by an aerated oxidation ditch for secondary treatment. Ferric sulphate dosing is used upstream of both the primary and secondary settlement tanks to assist precipitation of soluble phosphorus; sodium hydroxide dosing is used for pH adjustment downstream of the primary settlement tank. Treated effluent is discharged to a local burn. Sludge is taken to either WWTP_1 or another local treatment plant for processing therefore no final biosolids are produced at this plant.

WWTP_3: A medium sized municipal wastewater treatment plant receiving wastewater from domestic and commercial premises, including hotels and restaurants, and a large University and community hospital. There is no primary settlement at this plant, and the first stage of treatment is in an oxidation ditch dosed with return activated sludge, followed by secondary aeration (extended aeration), and final settlement. Final effluent is filtered through a sand filter and treated by UV radiation to deactivate pathogens. The effluent is discharged to the North Sea. Sludge dewatering is carried out using polyacrylamide dosing and centrifugation. The dewatered sludge cake is sent to landfill.

WWTP_4: A medium sized municipal wastewater treatment plant receiving a combination of storm water, and wastewater from domestic, commercial and industrial premises, as well as a small community hospital. There is currently no primary settlement used at this site, although a non-operational tank exists that was previously used for primary settlement. Effluent is introduced into a cyclic activated sludge system (CASS) in the form of a sequencing batch reactor (SBR) with four tanks. Each sequence takes place within a tank (filling, aeration, settlement and decanting), and a cycle is approximately 4-6 h, but can be operated on a reduced timescale at times of high flow if necessary. Ferric chloride addition is used for phosphorus removal, and polyacrylamide is used for sludge dewatering. Final sludge is removed by tanker and transferred to another facility (e.g. WWTP_1) for further treatment.

A certified reference material (CRM 055, Lot LRAA8035, *Sigma-Aldrich RTC*) was included in the study for comparison. The material, sourced from Canada, is stated to be sewage sludge from a domestic WWTP however the source plant configuration is not reported. The material has undergone thermal treatment (drying) and in this study will be defined as a biosolid. Certified reference values for TOC, metals and volatile solids for this material were obtained from the supplier.

6.3 Sample collection and transport

Of the four sites, only two (WWTP_1 and WWTP_2) included a primary settlement stage from which sludge solids were collected. Secondary sludge was collected from all four sites representing two nitrifying systems (WWTP_2 and WWTP_3), one non-nitrifying system (WWTP_1) and one partially nitrifying system (WWTP_4). All primary and secondary sludges were collected (where possible) before any chemical treatment such as lime addition or polymer addition before sludge dewatering. The return activated sludge in WWTP_4 contains a small quantity of coagulant (polyacrylamide), which is mixed in the activated sludge SBR, therefore settled solids are likely to contain a small quantity of coagulant although this is predicted to be low due to the high level of dilution. Sludge cake was collected from WWTP_3 and WWTP_4 and sludge pellets from WWTP_1. Dewatering in all these plants is assisted by polyacrylamide coagulant addition and untreated biosolids will contain a small dose of this compound.

Sludge samples were collected between April and July 2017 with details of the sampling conditions presented in Appendix 1. WWTP_1 was sampled on two separate dates, approximately 1 month apart (Apr and May) for primary and secondary sludge samples. Two batches of biosolid pellets were also collected from WWTP_1. One of these was pellets collected from the site the previous year (labelled “Old”) and one collected in April. Site operatives assisted in obtaining sludges from tanks at most sites due to health and safety concerns and restrictions on site access. Sample collection and handling followed common approaches and sampling protocols used elsewhere for pharmaceuticals and trace organics in environmental samples (e.g. Eljarrat and Barcelo 2004; Godfrey and Woessner 2004; Abegglen *et al.* 2009; da Silva *et al.* 2011; Al Aukidy *et al.* 2012). Sludge was collected using amber coloured, pre-labelled, 1 litre wide mouth bottles that had been pre-rinsed with ultra-pure water and air dried before sampling. Once gathered, the samples were collected from the site operatives and the date, time, a note of the weather conditions, and temperature and time of collection was recorded. A minimum of two litres of sludge was collected from each sampling point. Sludge is high in volatile solids, and water content may be between 70-80% by weight (Vergara *et al.* 2013), therefore enough wet sludge was required to ensure an adequate quantity of dry solids could be obtained. Samples were transferred into a cool-box and transferred directly to the laboratory where they were placed in a refrigerator at 4 ± 2°C until processed further.

6.4 Sample preparation

This section provides general sample processing details for sludge samples used throughout further experiments in this study. Samples were prepared for initial desorption experiments by multiple steps including sludge drying (settlement/dewatering, air drying), homogenisation and particle size reduction, and determination of moisture content.

6.4.1 Sludge drying

Immediately after collection, sludges were placed in a laboratory refrigerator for between 24 and 48 h to allow solids to settle out of suspension. Water was decanted off and discarded. The remaining settled solids were transferred onto pre-rinsed, labelled, foil-lined trays, placed on drying tables within a self-contained side room of the laboratory and lightly covered with paper towel (not in contact with sample surface) to avoid deposition of dust on sample surfaces. Samples were left to air dry at ambient temperature (20-24°C) as per method CEN/BT/TF 151 (10.2.3.2) (CEN 2007), which recommends application of samples in a layer not thicker than 5 cm. In this case samples were spread typically in layers of less than 1 cm to speed the drying process. Samples were kept away from any direct sunlight. Samples were turned regularly (at least daily) using a metal spatula to assist drying, and to prevent anaerobic zones become established in any of the trays. Time to achieve air drying varied by sludge type but typically took between 7 and 14 days.

6.4.2 Homogenisation and particle size reduction

Once samples were visibly dry, dried sludge was gently scraped off the foil liners into foil lined containers and stored out of direct light in a cool room for later use. Although the SPLP method does not specify a minimum particle size, it suggests a maximum particles size of < 9.5 mm. This meets the specification of most of the leaching tests and is more likely to apply to field applied biosolids, which would not be ground to a fine particle size as used in NEN 7341 and ASTM D 5744. The particle size for most dried sludge samples scraped off foil trays was well below < 9.5

mm as per the SPLP method, however, some samples required slight treatment by mortar and pestle to reduce larger pieces, such as sludge pellets and sludge cake. Each sample was transferred to foil lined containers for storage containers (e.g. foil lined glass jars) and stored out of direct sunlight for later use.

6.4.3 Determination of moisture content

Moisture content and volatile solid content were determined on a dry sub-sample for each sampling location. USEPA method 1684 was used for analysing the moisture content in the sludges and biosolids (EPA 2001). Samples were well mixed before sub-sampling and moisture content was determined in duplicate for each treatment plant/stage (apart from WWTP_2 where inadequate sample was available). If duplicates were found to differ by more than 10%, an additional replicate was taken for determination of the mean until all samples were within 10% of the mean.

The procedure for moisture content included the weighing out (in duplicate) of approximately 1 g of sample into a pre-dried, pre-weighed ceramic crucible (recorded to the nearest 0.0001 g) using a laboratory bench scale accurate to four decimal places. The crucible plus sample was then placed in a laboratory oven at 105°C (± 2 °C) for a minimum of 24 h. The crucibles were then removed from the oven and placed in a desiccator until cool (approximately 30 min) and reweighed. Moisture content (M_0) was then calculated as shown in Equation 1.

$$\text{\% Moisture } (M_0) = \frac{final\ weight\ (crucible + sample)}{Inital\ weight\ (crucible{+}sample)} \text{ x } 100$$

Equation 1 Calculation of moisture content

Results of moisture content are presented in Appendix 2. All replicates were found to be within 5% of each other.

6.4.4 Determination of volatile solids

Volatile solids determination was carried out following moisture content determination using a similar procedure as detailed for moisture content, but rather than using an oven to dry solids at 105°C, weighed samples and crucibles were placed in a laboratory muffle furnace at 550 °C for 4 h. Samples were allowed to cool inside the muffle furnace before being briefly stored in a desiccator before being reweighed to the nearest 0.0001 g.

The percentage of volatile solids was then calculated as the difference between the dry weight sample (total solid) following moisture content determination and the dry weight following ignition in the laboratory furnace as shown in Equation 2.

$$\text{\% Volatile solids (VS)} = \left(1- \frac{final\ weight\ after\ ignition(crucible + sample)}{final\ weight\ after\ moisture\ content\ \ (crucible{+}sample)}\right) \times 100$$

Equation 2 Calculation of volatile solids (%)

The results of the volatile solids tests are presented in Appendix 3.

6.5 Analytical Methods

The methods applied to each stage of the experiment will be present separately in subsequent chapters. However, analysis common to multiple experiments include pH and dissolved oxygen (DO) measurements and DOC determination. The methodology used in determination of these is presented here.

6.5.1 Determination of pH and dissolved oxygen (DO)

pH readings were determined using a *Hach*, Sension 3 handheld pH meter. Buffer solutions (*Reagecon* colour coded solutions pH 4.00, 7.00, 10.00) were used for instrument calculation and a separate buffer used to verify calibration (*Fisher Scientific* pH 7.00). A three-point calibration was carried out once per day (first use) according to manufacturer’s instructions and acceptance criteria. A check of pH 7.00 (± 0.05) using a second source buffer solution was carried out to ensure the calibration was accurate. pH determination on samples was carried out by removing the pH electrode from its electrolyte storage solution and rinsing with distilled water. The pH probe was placed in the sample, covering the electrode, and allowing the pH meter to stabilise. Once a stabilised reading was obtained, this was recorded to two decimal places. The accuracy of measurements is reported by the manufacturer as ± 0.2% of the pH reading.

For biodegradation tests, dissolved oxygen (DO) concentrations were measured in the aerobic and anaerobic tests using a *Hach*, Sension 6 laboratory DO meter. Probe calibration was carried out annually by the senior laboratory technician. The meter was turned on, and probe stabilised in ambient air. The sample probe was rinsed with distilled water before immersion in sample, and DO measurements recorded once a stable reading was reached. The sample probe was rinsed with DI water between samples.

6.5.2 Sample Filtration

Throughout the experiments, determination of DOC was carried out on samples that are filtered to separate solid materials and microbial interferences, and to capture only dissolved organic carbon fractions. All samples were filtered using 0.45 µm pore size filters (*PALL* GN-6 Metricel Grid, 47 mm). The basic filtration procedure is common throughout several experiments using the vacuum filtration apparatus as shown in Figure 6-2.

Figure 6-2 Sample filtration apparatus

There are some analytical considerations related to DOC contamination during filtration that should be noted. Khan and Subramania-Pillai (2006) found that many of the filter papers produced by the main manufacturers (e.g. Gelman, Whatman) of various compositions (cellulose acetate, glass fibre, nylon, polypropylene) were found to increase DOC concentrations in the filtrate. Some filter types were found to have no interference in the analysis of DOC, but pre-treatment of filter papers, by rinsing with 100-150 mL ultrapure water before sample introduction was found to reduce the potential transfer of DOC into samples from all filter paper types.

To reduce the potential transfer of DOC from filter papers, 150 mL of ultrapure water was filtered through filter papers before introduction of the sample. Samples were then filtered through the rinsed paper directly into sterile, rinsed amber coloured centrifuge tubes to minimising sample handling and potential for contamination, and stored in a refrigerator at 4 ± 2°C (for analysis or further processing within 24 h) or in a freezer at -18 ± 2°C (for analysis or further processing that would take place more than 24 h later).

6.5.3 DOC analysis

6.5.3.1 Detection of dissolved organic carbon (DOC)

DOC analysis was carried out on an *OI Analytical* model 1010 Wet Oxidation TOC Analyser fitted with model 1051 auto-sampler. The analysis of samples for DOC content is largely based upon the operating procedures provided by the system manufacturer (OI Analytical 2003). The analyser uses a persulfate oxidation method for analysis of samples up to 125 $mg{\cdot}L^{-1}$ organic carbon. The analyser introduces acid to the sample to be analysed, which causes carbonate and bicarbonate ions in the sample to be converted to CO_2. The release of CO_2 is purged by the system and carried into a nondispersive infrared (NDIR) detector, calibrated to display the mass of carbon dioxide detected. This mass is equivalent to the mass of total inorganic carbon (TIC). The concentration of TIC is measured as a ratio of this mass to the volume of sample analysed. After the system is purged of TIC, sodium persulfate is added, which reacts quickly with the organic carbon in the sample at 100°C to form CO_2, which is detected by NDIR. The detected mass of CO_2 is proportional to the mass of TOC in the sample, and the concentration determined as a ratio of mass to the volume of sample analysed. Experimentation was carried out to confirm the detection limit of the TOC analyser with the results presented in Appendix 4.

6.5.3.2 Sample analysis procedure

Reagents used in the operation of the *OI Analytical* 1010 TOC analyser included:

- Ultrapure water (*Puracel*, PURITE Select 18 MΩ)
- Sodium persulfate, $Na_2S_2O_8$ 100 g.L^{-1}; prepared by dissolving 100 g of $Na_2S_2O_8$ (*ACROS Organics*, 98+%) in 1 litre of ultrapure water. This was stored in a clean glass bottle and transferred to the analyser reagent bottle as required. Fresh solution is made up at minimum every three weeks, or more frequently if required.
- Phosphoric acid (5% vol/vol) prepared by adding 59 mL of *ACS* Reagent Grade (85%) H_3PO_4 to a 1000 mL volumetric flask, approximately half filled with ultrapure water, and made up to 1000 mL, using ultrapure water.
- Potassium hydrogen phthalate (KHP)
    - 1000 mg·L^{-1} stock solution was prepared by adding 2.128 g KHP (*BDH* AnalaR), previously dried at 110°C to constant mass into a 1 L volumetric flask and diluted to 1 L with ultrapure water. This solution was used to prepare calibration standards by dilution with ultrapure water. The stock standard and dilutions have a shelf life of three weeks.
    - Calibration standards of 1, 10, 25, 50, and 100 mg·L^{-1} were prepared by dilutions of the 1000 mg·L^{-1} stock solution with ultrapure water.
- Nitrogen gas (*BOC*, > 99.98% purity, 50-60 psi) was used for reagent purging.

An initial test run of samples was carried out to identify appropriate dilution levels to ensure sample results were within the calibration range of the analyser. This process identified that a 20 x dilution factor would be required for most samples to ensure solutions were within the instrument detection range of 1-100 mg·L^{-1}. Dilutions were carried out using ultrapure water.

6.5.3.3 TOC Analyser Operation

The analysis of samples was carried out according to equipment instructions. The analyser was set to stabilise for a minimum of 4 h prior to use or until NDIR detector was reading less than 10,000 counts as per manufacturer instructions. Reagent bottles (phosphoric acid and sodium persulfate) were filled at the beginning of each analytical run with fresh reagents (preparation described above), rinsing with a small quantity of reagent and discarding the rinse liquid. Nitrogen gas at 50-60 psi was turned on to enable reagent purging of CO_2. The sampling and nitrogen lines were added to each reagent bottle to confirm that the solutions in the bottles were being purged, as indicated by bubbling in the reagents. Pre-prepared (filtered and diluted) samples were loaded into an auto-sampler carousel in a pre-determined order starting with method blanks (filter blank, process reagent blank). An analyser reagent blank was included in the analysis by the TOC analyser as a measurement of carbon contamination in the reagents, gas, digestion vessel and tubing. Samples were arranged in the auto sampler in order of predicted low to high concentrations, where possible, to minimise carry-over effects. Dilution factors were entered into the equipment software for automatic calculation of final concentration. A reagent blank was included every 10 samples to detect any carryover, and to allow for a system rinse. Calibration was carried out using a five-point calibration (1, 10, 25, 50, and 100 $mg{\cdot}L^{-1}$ calibration standards).

6.5.4 Quality control

To maximise the ability to identify whether variation between sludge and biosolids types was due to factors such as plant type and/or treatment stage, the experiment has been designed to help ensure independence of measurements and observations by minimising the effect of random errors as much as possible. This included using standard operating procedures, ensuring instruments and devices were functioning as expected and calibrated, and that methods of sample handling, cleaning, the use of replicates, the use of blanks to identify contamination sources in the methods and analysis were used. For each new instrument calibration, the outputs of the calibration curve and R^2 value, indicating linearity of calibration, were reviewed. A limit of $R^2 \geq 0.98$ was used as the benchmark for calibration acceptance according to manufacturer’s instruction, and expert advice of the Abertay Senior Laboratory Technician. Calibration curves that did not meet the acceptance criteria were discarded and a new calibration was initiated. Outputs from the TOC analyser calibrations are included in Appendix 5.

Process interferences in the analysis of TOC can include contamination from vessels, reagents, water and tubing used in the experiment. This interference applies a positive bias to the results. Interferences of this nature were reduced by ensuring the use of high purity reagents or water within experiments and ensuring cleaning and rinsing procedures were carried out to minimise the likelihood of interference from glassware or tubing. Interferences can also occur in the calibration of the instrument. For samples with a low level of TOC, this can be significant, and may require a correction for background TOC in dilution water for the calibration standards. Interferences in the system due to non-CO_2 gasses in the NDIR detector is virtually nil as the detector is sensitised to CO_2 only and rejects responses from other gases. Interference from inorganic carbon (TIC) can be significant if the ratio of TIC:TOC is very high (e.g. 10 to 1). However, the result of the volatile solids test indicate that this is not the case for the samples assessed in this study. A summary of the quality control measures used included:

- *Leachate blanks*: Leachate blanks were included in each batch to check for contamination accrued through the extraction and sample filtration process. A summary of all leachate blanks is presented in Appendix 7.
- *Leachate replicates*: Three replicate of each sludge type were extracted under identical conditions.
- *Analytical replicates*: A duplicate measurement of TOC was carried out for each sample replicate, and a mean measurement recorded. All sample results were reviewed to check for agreement between analytical replicates. Where poor agreement was noted, samples were repeated.
- *Analytical blanks*: An analytical reagent blank (ultrapure water) was included in the analysis by the TOC analyser as a measurement of carbon contamination in the reagents, gas, digestion vessel and tubing. All analytical blanks were found to be at or near detection limit.
- *Calibration checks*: To ensure linearity of calibration, a limit of ≥ 0.98 was set for the calibration curve R^2 value. Calibrations not achieving this were repeated.

- *Statistical analysis*: To test for statistical differences between sludge types, techniques such as analysis of variance methods were used ($p < 0.05$, one-way ANOVA), multiple comparisons and non-parametric tests were used (Carballa *et al.* 2009). Statistical analysis software *IBM* SPSS (version 25, 2017) was used for the evaluation of results.

6.5.5 Limitations and areas of uncertainty

Despite the quality control measures employed above, there may be additional areas of uncertainty in the analytical processes used in this study. Some of these are described in the details of methods relating to specific experimental work presented in the relevant Chapters 7, 8 and 9. There are a few limitations of the study that may apply generally that are listed below.

1. Method of sample collection: Due to health and safety reasons, sample collection from treatment tanks at WWPT was carried out by onsite operatives. This was unavoidable but may present an area of uncertainty in the way samples were collected. At WWTP_1, samples were collected from tanks using a sampling valve used for sludge sampling. In contrast, samples collected at WWTP_2, 3 and 4 were collected using bucket sampling techniques. These factors may have impacted the relative location of sludge within treatment tanks (e.g. near surface, middle or bottom of the tank). This may account for some variability in the secondary tank samples.
2. Timing of sample collection: Timing of collection of samples from secondary tanks could also affect the composition of the solids collect depending on the timing of when the sample was collected (e.g. beginning of a cycle or end of a cycle) and could be another area of uncertainty in results for secondary sludges.
3. The sample particle size could be an area of uncertainty in the leaching tests. This is due to variations in how particles dried and were scraped off drying trays. Further study could be carried out to assess the effect of particle size on results.
4. General analytical error: As with any laboratory work, the potential for experimental error is present. This can include contamination of samples, mix up of labels, or dilution errors. To ensure this was minimised, good lab

practices of sample handling to minimise contamination was carried out. This included use of new or cleaned sample containers, rinsing of analytical equipment and apparatus between samples (e.g. pH and DO meters, filtration equipment). To reduce chance of sample mix-up or dilution errors, clear worksheets with labelling conventions, and dilution instructions were used when handling large numbers of samples.

CHAPTER 7 DETERMINATION OF DESORBABLE DISSOLVED ORGANIC CARBON (DDOC)

7.1 Introduction

The objective of this experiment was to determine the desorbable DOC (DDOC) from wastewater sludges and biosolids obtained from various types of WWTP. The experimental procedure draws on principles of solid waste leaching procedures used elsewhere as discussed in Chapter 4 (Washington State 2003; Morissette *et al.* 2015; Tiwari *et al.* 2015; USEPA 2017b)

7.2 Methodology

7.2.1 Theoretical background

Sorption to solids is an important factor in the movement and degradation of organic pollutants in the environment, determining the susceptibility of organic compounds to volatilisation, chemical or microbial degradation or to be leached into the environment (Wang and Jones 1994). Organic chemicals demonstrate various levels of association with solids in wastewater effluent, affected by hydrophobic partitioning, ion exchange, complexation and hydrogen bonding as described in detail in Chapter 2 (Delle Site 2001; da Silva *et al.* 2011). Barret *et al.* (2012) described the concept of micropollutant partitioning in wastewater to comprise of three phases including dissolved in the aqueous phase, sorbed to particles or sorbed to dissolved and colloidal matter (DCM) as depicted in Figure 7-1.

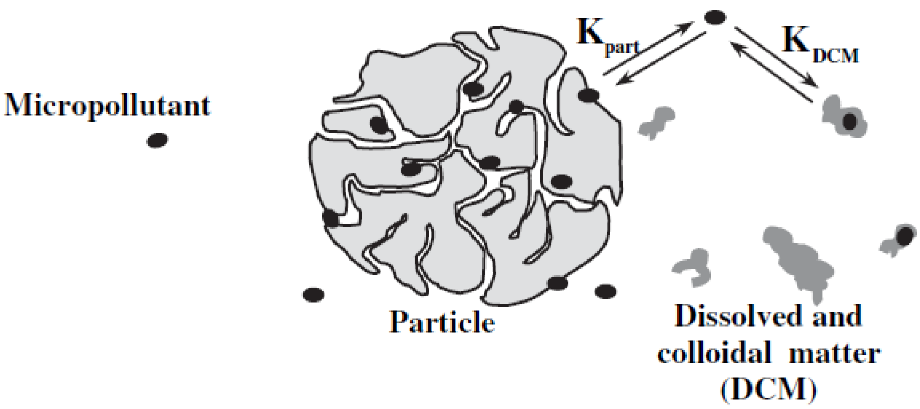


Figure 7-1 Three compartment concept of micropollutant partitioning in wastewater (Barret et al. 2012)

This description of organic pollutant partitioning helps to explain why POP removal in a WWTP cannot be fully explained by basic sorption and settlement processes during primary treatment and biodegradation processes during secondary treatment. Treatment stages may accumulate different quantities and types of compounds in their sludge. Hydrophobic and hydrophilic compounds in the dissolved fraction can potentially move between treatment stages if associated with DCM and POPs may not be irreversibly bound to particles or DCM. Operational factors such as pH, hydraulic retention time (HRT) or solids retention time (SRT) may also affect the sorption, desorption and transport of compounds through the treatment system, as discussed in Chapter 2. Final treatment of sludges by dewatering, AD or thermal treatment may also impact the quantity and types of POPs that may remain at the end of a treatment process. There have been few studies that have investigated the concentrations of POPs in sludges at different stages of the wastewater treatment process or biosolids treated by different techniques (Horsing *et al*. 2011; Martin *et al*. 2012). These studies have focussed on absolute concentration determination and have not assessed the relative desorbability of POPs from sludges.

Current sludges management processes typically bulk sludges from all stages of the wastewater treatment processes together to produce a mixed biosolid for land application. There has only been limited consideration of how each treatment stage may contribute to the overall biosolid pollutant burden. Further study that can therefore improve the understanding of pollutant partitioning at various stages may improve understanding of appropriate treatment processes to reduce POP burden in sludges. The leaching process used in this experiment also helps to quantify DOC fluxes from sludges and biosolids, which may be an important indicator of direct pollutant transfer to the environment or transfer of pollutants including POPs and trace metals sorbed to DOC, thereby improving understanding of environmental risk from land application of various types of municipal wastewater treatment sludges and biosolids.

7.3 Leaching procedure

In this study the modified USEPA synthetic precipitation leaching procedure (SPLP) SW 846 Method 1312 described in Chapter 4 was selected for determination of DDOC from various biosolids for the reasons previously discussed (e.g. the use of a water-based solvent, exclusion of interferences for DOC analysis, a method validated elsewhere as suitable for use on municipal wastewater sludge/biosolid materials and suitable for evaluation of leachability of non-volatile organic compounds). The modified method was developed based on scaled down sample mass and leaching fluid volumes. An experiment to examine the effect of two variables, pH and shaking time, was carried out to assess the effectiveness of the prescribed method for determining leachable DOC from a biosolids matrix. The results are presented in Appendix 6.

The method used in this study did not include the use of zero headspace vessels, which would be used to determine volatile organics. Most volatile organics should have been removed during sample handling, drying and processing and this portion of DOC was not assessed. In this study, volatile compounds were excluded from consideration, given they are more likely to dissipate naturally over time, and less likely to be transported into the environment through processes of leaching. Volatile solids however were determined for each sludge type, as presented in Appendix 3.

7.3.1 Leaching fluid preparation

A stock solution of sulphuric acid and nitric acid at a 60:40 volume/volume ratio was prepared by adding 30 mL of sulphuric acid (2.5 M) and 20 mL of nitric acid (2.5 M) (both *Fisher,* Laboratory Reagent grade) into a small laboratory flask. The mixture was carefully mixed to ensure a homogenous solution and then added dropwise using a glass pipette into a 2 L glass jar, filled to near the 2 L mark with ultrapure water. A calibrated pH meter was used to measure the pH, as described in Chapter 6 after each addition of acid until the pH reached a value of 4.20 ± 0.05 pH units. The leaching fluid was then used directly for leaching experiments and subsequently stored in a refrigerator at 4 ± 2°C until ready for use. If stored in the refrigerator, the leaching fluid was removed from the refrigerator in advance of leaching procedures and allowed to reach room temperature before use. The pH was checked once leaching fluid reached room temperature and if found to be above or below 4.20 ± 0.05 pH units, either more of the sulphuric/nitric acid solution was added, or more ultrapure water was added to decrease or increase the pH respectively.

7.3.2 Sample preparation

New sterile amber coloured centrifuge tubes and caps were pre-rinsed with a small quantity of ultrapure water and air-dried upside down in a laminar flow cupboard. Tubes were labelled with appropriate sample site/process and replicate number, with four tubes prepared per site to allow for three replicates plus one spiked sample per sludge/biosolid variant. The amber tubes were used to minimise the possible interference of light on photo-degradation of organic compounds within the sludge. Initially A 20:1 liquid to mass ratio of leaching fluid to biosolid sample was prepared, as prescribed in the method, by adding approximately 2.5 g dry weight (dw) of biosolid sample to each tube, made up to 50 mL with leaching fluid, with samples prepared as described in Chapter 6. Initial experimental working found that a 2.5 g sample size resulted in solutions with DOC concentrations that significantly exceeded the analytical range of the TOC analyser requiring additional dilution of samples ranging from 50-100 vol/vol with water. To reduce the scale of dilution factors required, a reduced mass of 1.0 g was used for subsequent samples. Sample mass was recorded to four decimal places as wet weight, and moisture content, as calculated in Chapter 6, was used to calculate the dry weight mass in each sample, as recorded in Appendix 7.

One matrix spike (2.0 mL of 1000 $mg{\cdot}L^{-1}$ KHP) per site was added to one replicate before making the sample up to 50 mL with ultrapure water, to give an approximate concentration of 40 $mg{\cdot}L^{-1}$. One reagent blank (leaching fluid) was included per batch of 10 samples. In addition, four tubes (three replicates plus one matrix spike) were prepared as per samples for the reference sludge material (CRM055). Details of blanks and spikes prepared and detected concentrations of DOC are listed in Appendix 7.

7.3.3 Extraction procedure

Sample tubes were tightly capped and loaded onto the mixing apparatus (Figure 7-2) to be turned end-over-end at a rotation of 30 ± 2 rpm for 18 ± 2 h at room temperature (e.g. 23 ± 2°C). Extraction dates and start times and end times were recorded (Appendix 7). At the end of the rotation procedure, samples were arranged in a sample rack to allow solids to settle before filtration. A trial run of the procedure found that filtration of the leachates using 0.45 μm pore filters was extremely difficult after 30 minutes settlement, therefore, samples were subsequently centrifuged for 5 minutes at 300 rpm to assist filtration. Sample pH was recorded following centrifuging using a handheld pH meter (*Hach*, Sension 3) (Appendix 7).

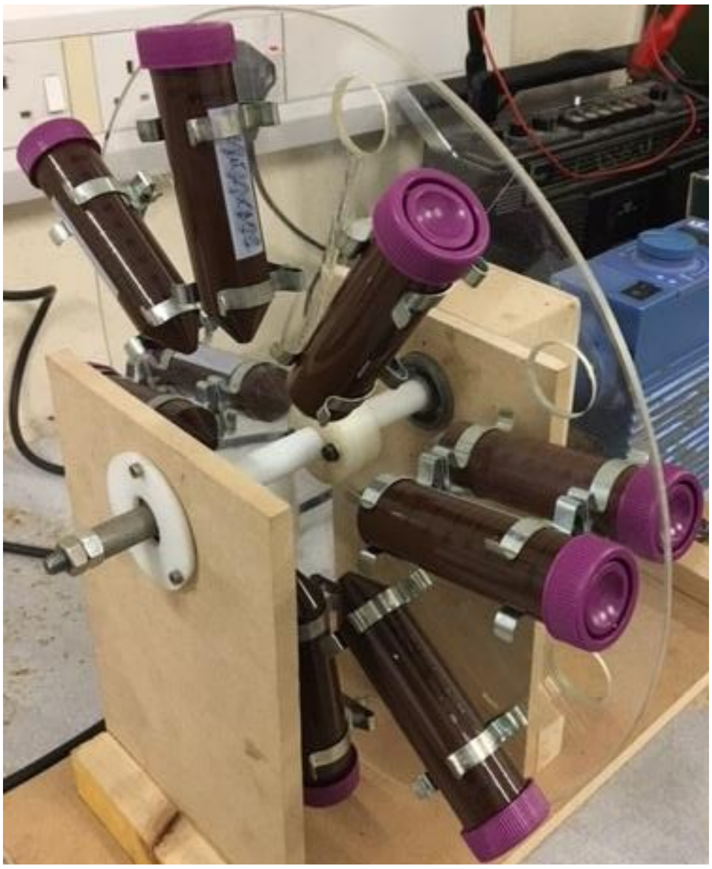


Figure 7-2 Extraction apparatus

Samples were filtered using vacuum filtration and 0.45 μm filter papers (PALL GN-6 Metricel Grid, 47 mm) and stored in a refrigerator at 4 +/- 2 °C (for analysis within 24 h) or in a freezer at -18 +/- 2 °C (for analysis that would take place more than 24 h later).

7.3.4 Determination of DDOC in leachates

The filtered fraction of samples leachates was assumed to contain the maximum quantity of DDOC possible to extract using the SPLP method. The DDOC was determined for all samples using the methods described in Chapter 6. Results from the TOC analyser were reported as $mg \cdot L^{-1}$ with dilution factors applied using the TOC data analysis software. These values were converted to $mg \cdot kg^{-1}$ dw using initial sample mass, extraction volume, and moisture content using Equation 3.

$$DDOC = DOC_i \left[\frac{V_i}{W_w(1 - M_o)}\right]$$

Where:

DDOC = desorbable dissolved organic carbon (mg.kg^{-1})

DOC$_i$ = TOC concentration measured in leachate, with dilution factor (mg·L^{-1})

V$_i$ = sample volume (L)

W$_w$ = wet weight (mg)

M$_o$ = moisture content of the sample, as expressed in decimal format

Equation 3 Calculation of desorbable, dissolved organic carbon (DDOC) in mg·kg^{-1}

Full results showing raw data and calculated DDOC are presented in Appendix 7. All batch blanks were found to be below detection limit after sample dilution.

7.3.5 Determination of Pb and Cd concentrations in leachate

For a selection of samples, leachates analysed for DDOC were also analysed for presence of trace metals, to identify whether a correlation between the flux of DOC and inorganic compounds would be identified, as suggested by the literature. A spectrophotometer (*Hach-Lange* DR 5000) and cuvette kit tests for cadmium (Cd) (*Hach-Laing* LCK 308; detection range 0.02 – 0.3 mg·L^{-1}) and lead (Pb) (*Hach-Laing* LCK 306; detection range 0.1-2.0 mg·L^{-1}) were used on undiluted leachates.

7.4 Results

7.4.1 Mean DDOC

Mean DDOC (mg·kg^{-1} dw) ranged from 11,760 to 33,853 mg·kg^{-1} across sludge and biosolid types. The mean DDOC values for each WWTP and treatment stage are shown in Table 7-1, and illustrated in Figure 7-3 with error bars representing two standard deviations (SD).

Table 7-1 Mean Desorbed Dissolved Organic Carbon (DDOC, mg·kg^{-1}) from wastewater sludges extracted using the modified SPLP leaching procedure.

Sample site	Sludge type	Mean DDOC (mg·kg^{-1})	SD	N
1	Primary (Apr)	21,692	756	4
1	Secondary (Apr)	22,864	1,011	4
1	Biosolid (Apr)	22,542	2,359	2
1	Primary (May)	21,985	3,128	4
1	Secondary (May)	33,853	1,574	3
1	Biosolid (old)	27,862	1,741	3
2	Primary	26,537	1,519	4
2	Secondary	23,054	454	3
3	Secondary	11,760	994	3
3	Biosolid	29,983	1,159	3
4	Secondary	20,275	1,089	3
4	Biosolid	26,155	1,408	4
Reference	Biosolid	14,422	323	3

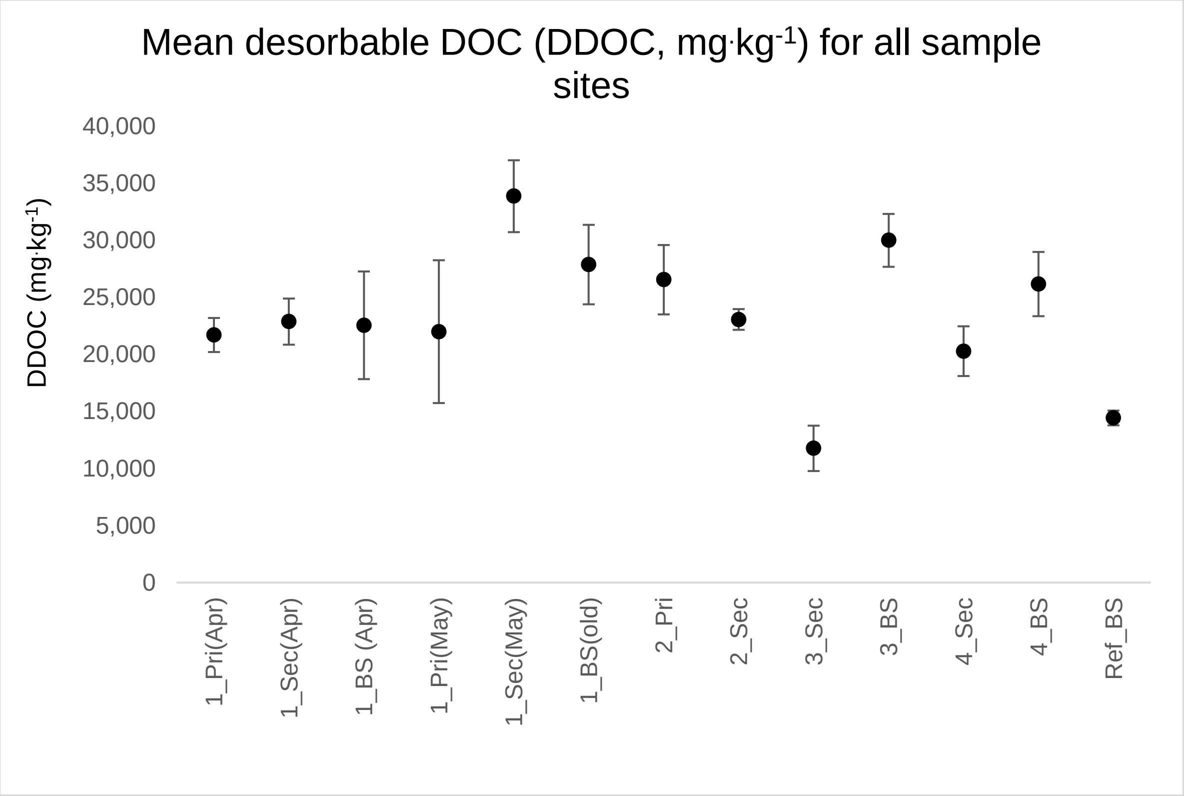


Figure 7-3 Mean DDOC by treatment plant and treatment stage (error bars showing 2 x SD)

As shown in Figure 7-3 the variability of results was found to be relatively small for some samples (e.g. reference material) and large for others (WWTP_1 primary, May). This may have been due to uniformity of sample particle size, or due to interferences in the analysis for some samples. The primary sludge samples from WWTP_1 for example varied between the April and the May sampling, although mean results were very close. In contrast the secondary samples in April and May did not show as great a difference in variability, however, means were higher in the May sample. This may have been due to less homogenous subsampling of the May sample. The higher variability in the biosolids samples may have been due to the difference in particle size, compared to some of the other sludges that have more regular particle size. For example, the reference biosolid, which was presented as a ground powder showed little variability in results.

A visual presentation of results compared by treatment stage (primary, secondary and biosolid) is presented in Figure 7-4. Although there are many variables across the types of sludges included in each result, the boxplot suggests that across treatment plants, there is only a small observable difference in mean DDOC between primary and secondary sludges, although the variability is high for secondary sludges and the range of results is high. Differences in the types of secondary treatment processes assessed, and the difference in sample collection practices or timing during a treatment cycle could account for this variability. In general, the results for biosolids had a higher mean DDOC than the other sludge types, although this also varied widely depending on the source treatment plant. The small set of outliers in Figure 7-4 represents the results from the reference material (CRM 055). This material underwent thermal treatment, which may account for the loss of readily desorbable DOC, and notably lower mean DDOC compared to the biosolids from WWTP_1, 3 and 4.

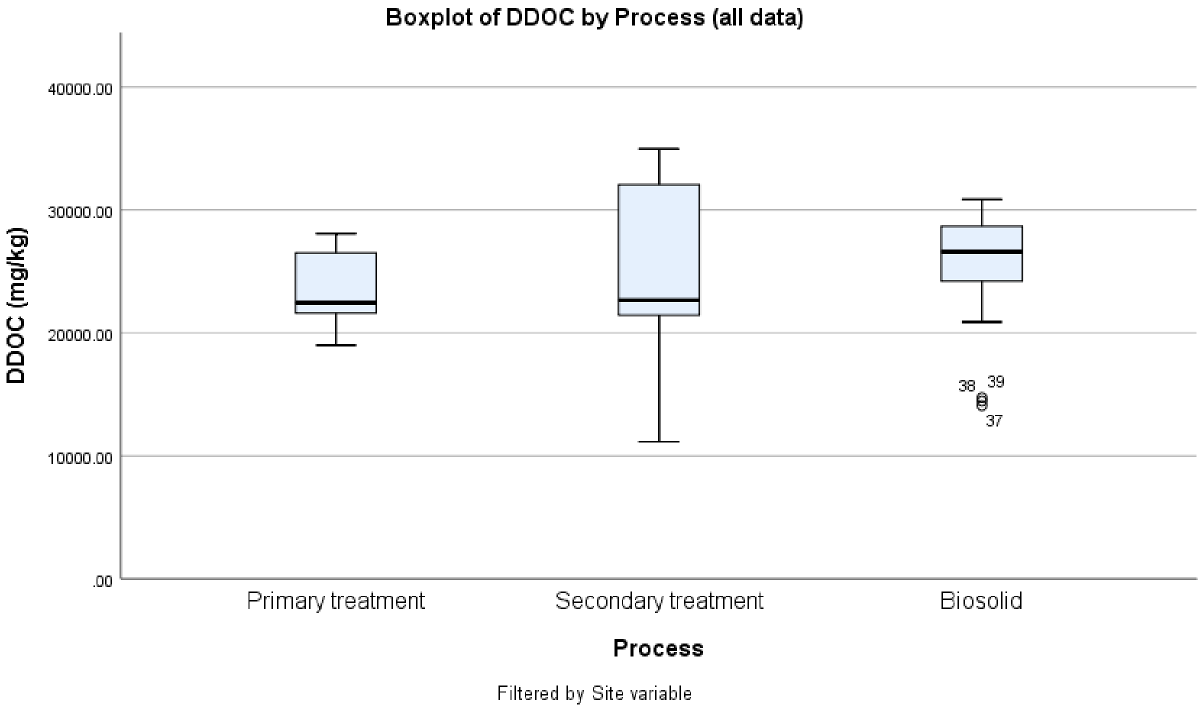


Figure 7-4 Comparison of results by sludge from three treatment stages (primary treatment, secondary treatment and biosolids)

Further examination of the results is presented in Figure 7-5 showing mean results for process type by each site. The primary sites (graph A) show relatively similar results, despite the variability between the April and May samples. There are more observed differences between the secondary samples (graph B) and the biosolids (graph C).

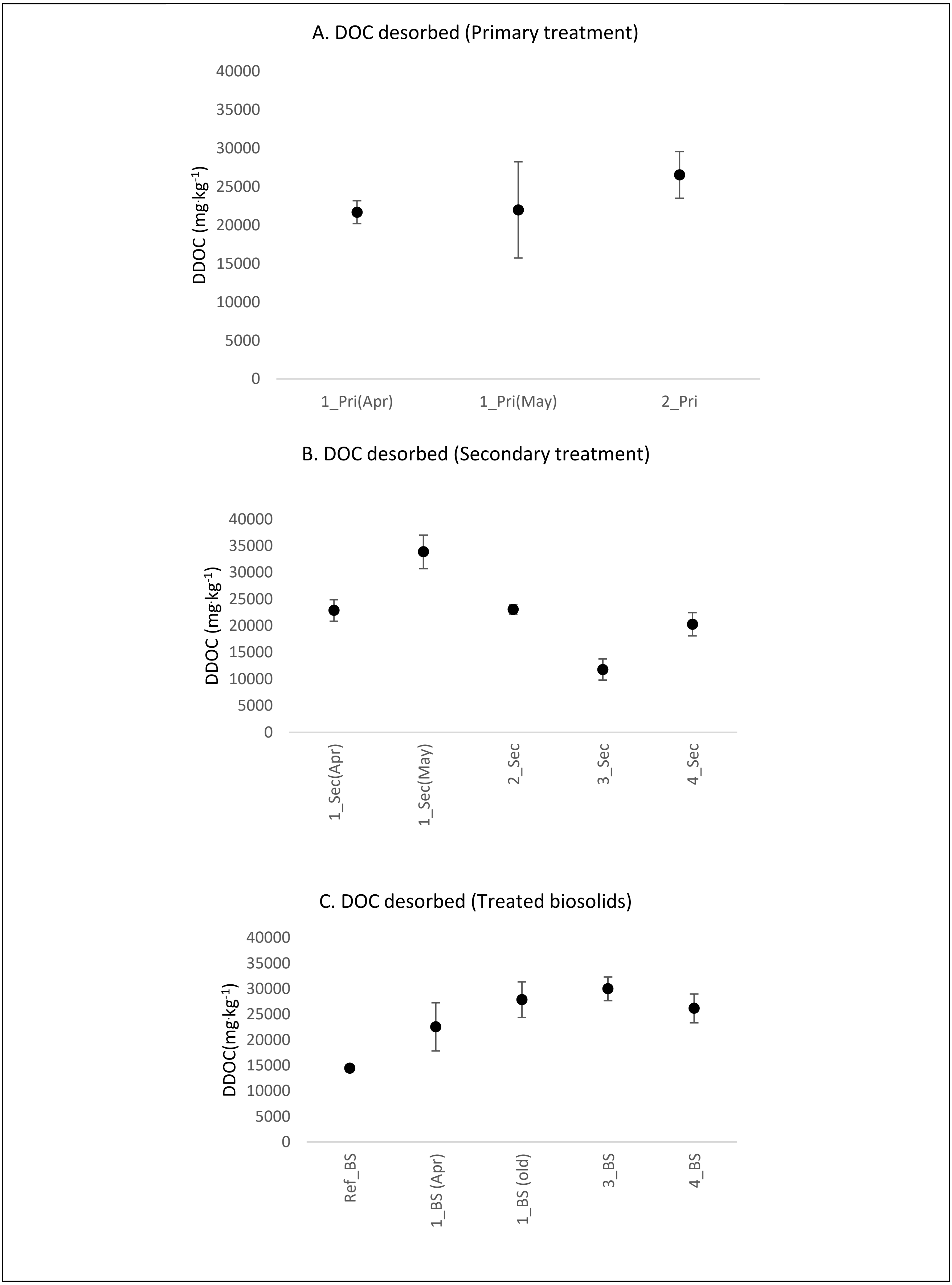


Figure 7-5 Comparison of DDOC by site for each sludge type (A. Primary, B. Secondary, C. Treated biosolids)

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Both Figure 7-4 and Figure 7-5 indicate that most of the biosolids leached between 20,000 and 30,000 mg.kg^{-1} DDOC. Greater variability is observed between sites for secondary sludges and biosolids. Less variability is detected between sites for the primary treatment sludges. The reasons for this may be related to the small number of primary sludges obtained (only two sites) but also may be due to the primary treatment stage offering little variation in the type of treatment (e.g. settlement only), which typically does not differ between treatment plants. In contrast, secondary sludges were collected from all four WWTP and process conditions varied between these. To examine this further, results were compared for secondary sludges from plants with different levels of nitrification (nitrifying, non-nitrifying, and partially nitrifying), with a boxplot of results presented in Figure 7-6.

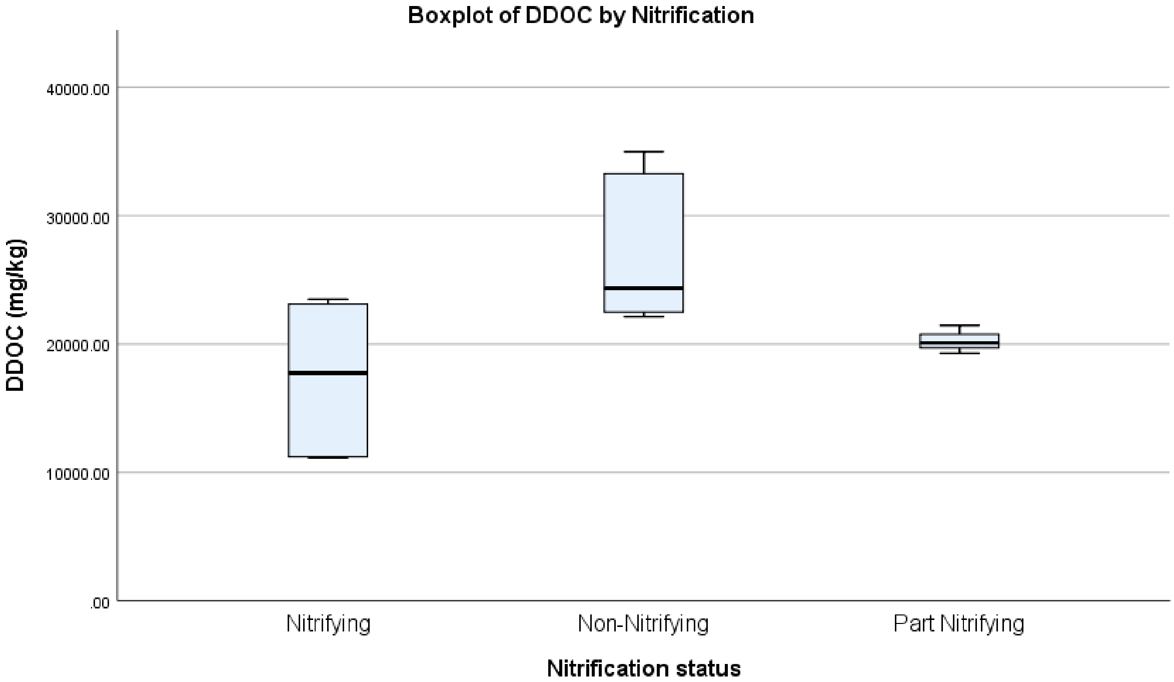


Figure 7-6 Boxplot of DDOC results by nitrification type (secondary sludges from all sites)

Figure 7-6 suggests a higher concentration of DDOC was leached from the non-nitrifying plant as compared to the nitrifying and partially nitrifying plants. The nitrifying plants have a longer HRT (18-27 h), whereas the non-nitrifying plant has an HRT of just 4 h. Extended aeration at the nitrifying plant is likely to result in more biodegradable organic carbon being consumed, due to co-metabolism by ammonia oxidising bacteria (AOB) as discussed in Chapter 2 (Xu *et al.* 2016). The SRTs were not available for WWTP_1 and WWTP_4, but for the nitrifying plants, it was estimated at about 24 d at WWTP_1 and 6 d at WWTP_3. These plants had similar HRTs. WWTP_3 secondary sludge was found to have a lower DDOC than WWTP_2, but there is not enough evidence to suggest that differences were due to SRT.

A statistical comparison of means was carried out to identify whether statistically significant differences in DDOC were observed for treatment stage or nitrification status. Results were analysed using SPSS and are presented in Table 7-2. Analysis of variance (ANOVA) indicates that there are significant differences in DDOC based on site (WWTP_1 and WWTP_2 differ from the Reference material), and multiple comparison tests indicate the direction of the difference. The analysis found no significant difference between different process stages, but significant differences were found between nitrification for secondary sludges (nitrifying and non-nitrifying plants).

Table 7-2 Summary of analysis of variance (ANOVA) and comparison of means

Variable	Significance (ANOVA)	Multiple comparison tests
Wastewater treatment plant	0.024 (Reject null-hypothesis	Post Hoc Test (Tukey HSD) WWTP_1 > Ref (p = 0.020) WWTP_2 > Ref (p = 0.039) Other comparisons p > 0.05
Process stage	0.618 (Retain null-hypothesis)	All comparisons p > 0.05
Nitrification (assessed on secondary sludges only)	0.018 (Reject null-hypothesis)	Nitrifying < Non-Nitrifying (p = 0.016) Other comparisons p > 0.05

Null hypothesis: There is no difference between treatments

7.4.2 Trace metals and DDOC

Several sludge leachates were used for trace metals screening to assess whether any correlation could be observed between DDOC concentrations and trace metals concentration. Leachates from WWTP_1 and WWTP_3 (all sludges and biosolids), and the Reference Material were analysed for dissolved Pb and Cd and compared to results for DDOC in solution (mg·L^{-1}).

All samples were found to have low or non-detectable concentrations of Cd and Pb. For Cd, all samples were found to be below the detection range for the instrumentation and test method used, therefore examination for correlation with DDOC results was not possible. For Pb, many of the results were near the detection limit for Pb. Figure 7-7 presents the Pb results in relation to measured DDOC results.

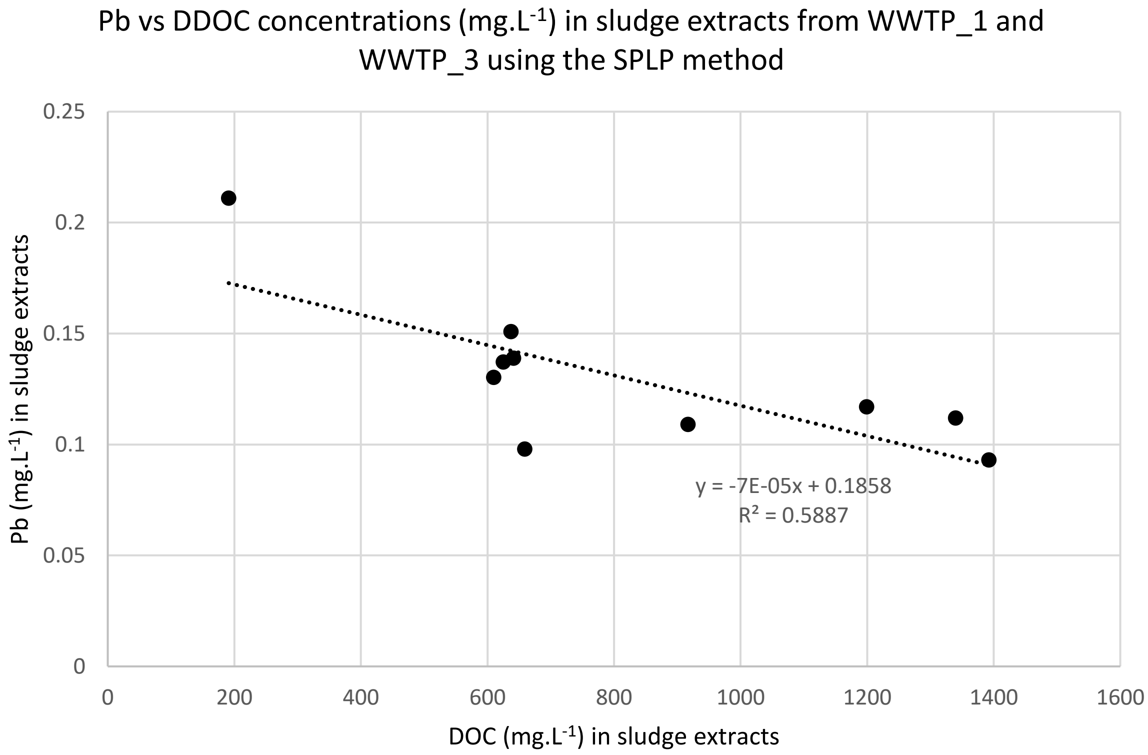


Figure 7-7 Pb vs DDOC concentrations in sludge leachates

The results present some indication that DDOC may be negatively correlated with Pb concentration however, with results all close to method detection limit, the conclusions are not clear and a poor R^2 value is given, indicating poor linearity. A bivariate correlation analysis was carried out using SPSS, with data presented in Katsoyiannis and Samaras (2007) also found a negative correlation between DOC concentration and Pb (-0.47) and Qi *et al.* (2011) also noted that in sludge/lignite amended soils Pb leaching was reduced, suggesting that a high total organic carbon content reduces leaching.

Table 7-3. A relatively high negative correlation is indicated by the Pearson's correlation (-0.767), and the result is significant with p = 0.010. The highest result observed was for WWTP_3 secondary sludge. This result was more than double the result for the other samples, but there is no known reason why this plant should have a higher level of Pb in the leachate, although there may be features of the contributing catchment that could be responsible. This may include the high density of older and historical buildings that are more likely to have lead piping. The negative correlation between DDOC and Pb suggests that a lower level of organic carbon in the sample, in general, may be linked to greater leaching capacity in the sample (fewer binding sites for metals). If the high result is removed however, the strength of the correlation between DDOC and Pb is reduced and the significance value increases to 0.066, outside the significance range of $p < 0.05$. Katsoyiannis and Samaras (2007) also found a negative correlation between DOC concentration and Pb (-0.47) and Qi *et al.* (2011) also noted that in sludge/lignite amended soils Pb leaching was reduced, suggesting that a high total organic carbon content reduces leaching.

Table 7-3 Analysis of correlation between DOC and Pb concentration in selected leachates

	All data	**[High value removed]**
Pearson's Correlation	-0.767	-0.635
Significance (2-tailed)	0.010	0.066
N	10	9

Four replicates of the reference material CRM 055 were included in the screening experiment, with a mean leachable Pb concentration calculated to be 3.05 $mg{\cdot}kg^{-1}$ dw (SD: 0.21). The certified concentration of Pb in the sample is 154 ± 12.4 $mg{\cdot}kg^{-1}$ suggesting approximately 2% of the Pb in the sample was leached using the SPLP method. This is a relatively higher leaching rate in comparison to a study using the SPLP method on lead contaminated soils carried out by Pinto and Al-Abed (2017). In Pinto and Al-Abed (2017) a contaminated soil with approximate Pb levels of 4400 $mg{\cdot}kg^{-1}$ was found to leach approximately 0.2 $mg{\cdot}L^{-1}$, or roughly double that of the CRM in this study, despite containing about 25 times more lead in the raw material. A major difference in the soils and the biosolids in this study was the % TOC, which was about 2.8% for the contaminated soil compared to nearly 30% for the CRM in this study. This comparison is contrary to the negative correlation found in this study and tends to suggest that the higher TOC in this study could contribute to relatively higher leaching levels, however there are several other unknown variables in the compositions of the soils to make a definitive comparison with the soil study. For example, although total TOC was reported, the Pinto and Al-Abed (2017) study did not measure DDOC, which may have improved comparison of these studies. In addition, other soil properties such as presence of inorganic constituents may have reduced Pb leaching. In any case the results suggest that further study on the influence of DOC on transport of metals into the environment from biosolids is needed.

7.4.3 Volatile solids and DDOC

The volatile solid (VS) content was determined for each sample site and sludge type as described in Chapter 6, with data reported in Appendix 3Appendix 7. The VS results were compared to DDOC to identify if VS provides a good predictor of desorbable organic carbon. The results are shown in Figure 7-8, with DDOC results for test sites and the reference material, arranged from lowest to highest, and relative VS results overlaid. Results were arranged in this manner to provide a visual assessment of whether VS results would also show an arrangement from lowest to highest, but this was not observed. To confirm this, SPSS was used to calculate correlation coefficients for mean DDOC and mean VS. The Pearson correlation was determined to be 0.138, with a two-tailed significance value of $p = 0.703$. These results suggest a poor correlation between DDOC and VS.

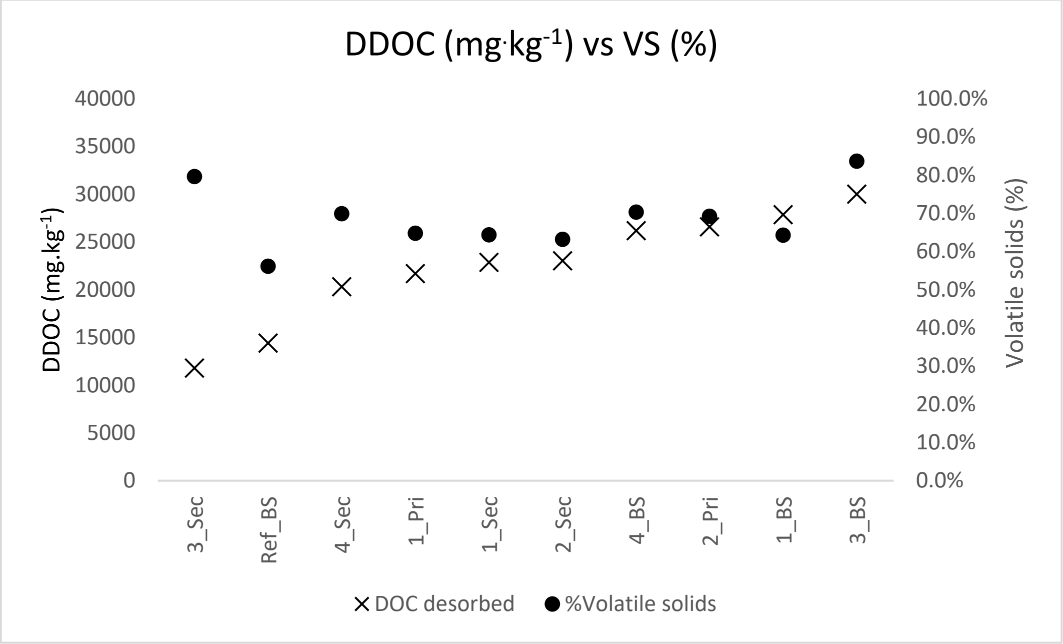


Figure 7-8 Comparison of mean DDOC and volatile solids

The secondary sludge from WWTP_3 again stands out as somewhat of an outlier compared to the other samples. The high VS result and low DDOC may indicate that this sample has a high concentration of microbial matter that was filtered out during the sample processing stage. This may be due to the sample being collected during an active stage of microbial growth within the activated sludge reactor.

7.5 Discussion

7.5.1 DDOC extraction procedure

The study has presented baseline data on the quantity of DDOC using the SPLP method. Comparing mean DDOC for the CRM reference material (14,422 mg·kg^{-1}) and the certified total organic carbon content of the material (29.9 Wt%), the quantity of DOC desorbed represents 4.8% of the total organic carbon present in the sample. In comparison to other studies that have measured leachable DOC from wastewater sludges, this study finds a greater concentration of leached DOC. For example Wijesekara *et al.* (2017) estimated desorption of DOC from biosolids (WWTP sludges after aerobic treatment and AD) using an alternative method (shaking of samples with water for 30 minutes at 30 rpm, followed by 30 minutes centrifuging), with results of 2,815 mg·kg^{-1} and 5,635 mg·kg^{-1} for samples with TOC (Wt%) of

45.3% and 35.2% respectively. These results are between 2 and 12-fold lower than the range of DDOC measured in this experiment (11,760 mg·kg^{-1} to 33,853 mg·kg^{-1}) and indicate a DDOC of 0.6% and 1.6% of TOC respectively (Wijesekara *et al.* 2017). Ashworth and Alloway (2004) used a method similar to the SPLP method (1:5 ratio of sludge to water, shaken for 24 h) with the primary differences being the shaking speed (14 rpm vs 32 rpm) and the extraction fluid (neutral water vs pH adjusted). The study found that the quantity of DOC leached from anaerobically digested sludges, with a 27 Wt% organic carbon content, was 4,395 mg·kg^{-1}, or approximately 1.6% of TOC. This result is also lower than the results obtained in this study. The preliminary experiment of the effect of pH and shaking time undertaken in this study indicated that starting pH did not appear to have a significant effect on DDOC but shaking time did. The effect of pH on DDOC is shown in Figure 7-9 and the effect of shaking time is shown in Figure 7-10 with a shaking time between 18 and 24 h achieving the maximum leachate DDOC concentration. Full results of the pH and shaking time study are presented in Appendix 6.

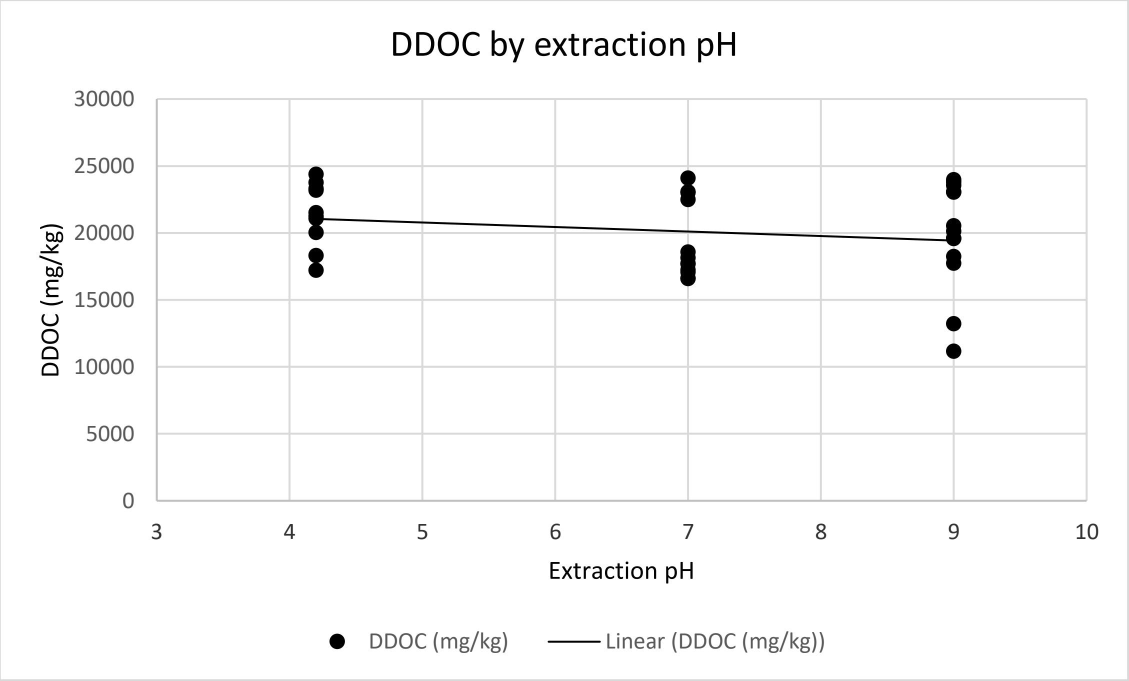


Figure 7-9 Comparison of effect of pH on DDOC

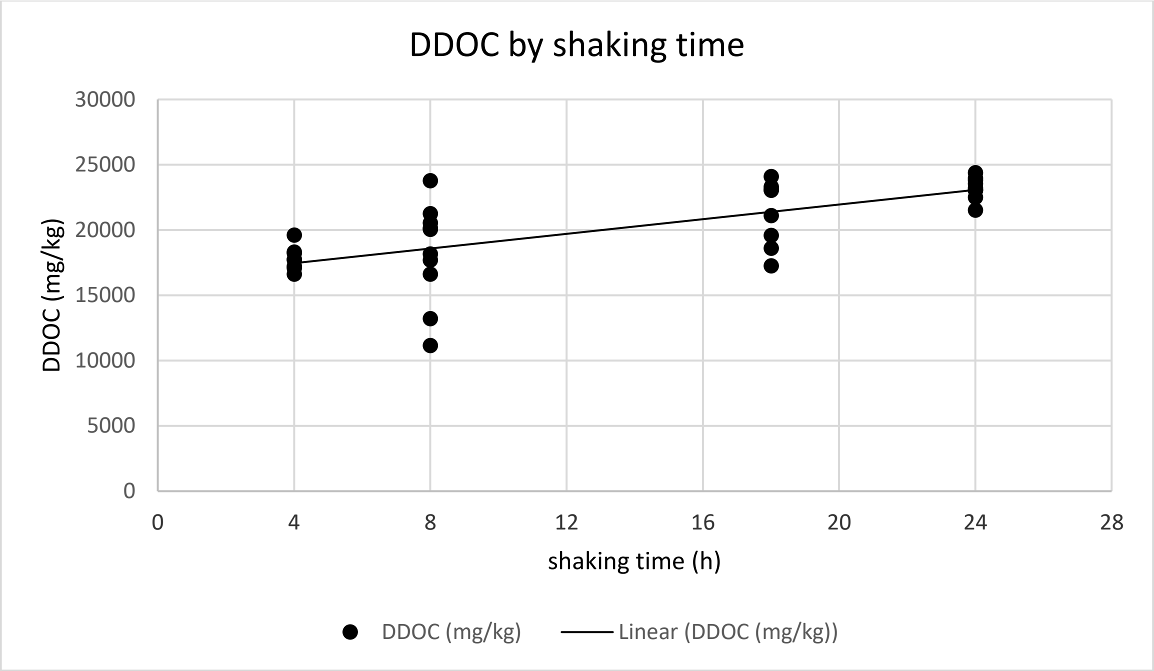


Figure 7-10 Comparison of the effect of shaking time on DDOC

The results obtained by Wijesekara *et al.* (2017) had a significantly reduced shaking time compared to this study (30 min vs 18 h), which may account for the difference. In addition, the 30 minute centrifuging process may have also reduced some DDOC if DCM was removed from suspension through centrifuging. The differences in measured DOC between this study and the Ashworth and Alloway (2004) study are slightly more difficult to explain. The use of an extraction fluid with neutral pH is unlikely to be responsible for the difference, as the study of the effect of pH and shaking time indicated that starting pH (4.2 and 7) did not result in a significantly different DDOC. In this case, the reduced shaking speed may account for some of the difference. This study found that despite using an extraction fluid of pH 4.2, the final pH of all samples significantly increased over the study period. The final pH does not appear to be correlated with the DDOC values obtained, however further investigations to characterise DDOC could examine whether this pH difference is correlated with the type of organic carbon that has desorbed, as pH is shown to have an impact on dissociation processes, and hence relative levels of sorption for some organic compounds (Sigmund *et al.* 2015). In this study, the final sample pH ranged from 6.11 to 7.43, suggesting that neutralisation of acids in the samples resulted in increasing pH over the extraction period. The samples with the lowest final pH were from WWTP_2, primary settled sludge and the highest were from WWTP_1, primary settled sludge. Wang *et al*. (2018) examined the influence of pH on the mobility of six POPs to examine how hydrophobicity is affected and interactions between the organic pollutants and dissolved organic matter. Leachability varied by compound type, with maximum leachability at neutral pH (7.5) for some and high pH (9.5) for others, but DOC content appeared to have a reduced influence at lower pH.

7.5.2 Discussion of process factors affecting the fate of DOC

The predicted fate of organic pollutants in treatment works includes sorption to particulate matter, sedimentation, some volatilisation and minimal biotransformation in the primary treatment stage, and air stripping and biodegradation in the activated sludge stage (Byrns 2001). These predictions assume that organic pollutants in the primary treatment stage are largely available for sorption to solids, and in the secondary treatment stage, are largely available for biodegradation. However, the presence of a third phase (sorption to dissolved and colloidal matter DCM) and its importance in pollutant transport as proposed by Barret *et al.* (2012) suggest that predictions on contaminant partitioning between various wastewater treatment stages may not be so straightforward. This study found that there was not a statistical difference in DDOC between treatment stages. There was higher variability in the secondary treatment sludges compared to primary treatment sludges and biosolids, although there were only two sites with primary sludges compared to all sites with secondary sludges. The results suggest that all biosolids will leach DDOC at a similar magnitude, between 20,000 and 30,000 mg·kg^{-1}. Further stages of this study will examine how the leachates differ. Differences observed between secondary sludges from nitrifying vs non-nitrifying plants indicate that there may be an influence of process parameters on DDOC. Secondary sludges for plants employing nitrification with longer HRTs were lower than for the non-nitrifying plant, suggesting that the extended aeration at nitrifying plants may be important in DOC reduction, possibly through the co-metabolism by AOB. Semblante *et al.* (2015) reviewed removal efficiency and influence of wastewater treatment processes on the removal of trace organic compounds and found that aerobic processes have a greater potential to enhance biodegradation. Within treatment processes, control of dissolved oxygen concentrations may be important to biodegradation, as will enhanced retention time to facilitate biological breakdown. There is evidence in this study that nitrifying plants with higher HRTs demonstrate greater potential for reduction of DOC, which supports this suggestion.

The study found that the DDOC from biosolids that had undergone additional treatment varied by treatment type. The biosolids that had undergone minimal processing (e.g. dewatering only) from WWTP_3 and WWTP_4 had higher levels of DDOC than those from WWTP_1, which had undergone AD treatment, and from the reference material, which had been thermally treated. The DDOC of the reference material was much lower than the other biosolids, indicating that the thermal treatment process may have reduced the total organic carbon available for desorption. Further characterisation of the DOC obtained from each of the treatment processes in this study will allow for greater evaluation of the effects of process on the persistence of the desorbed organic carbon compounds found in the study.

7.5.3 Discussion of the influence of DOC on the movement of other pollutants

The study found poor correlation between volatile solids and DDOC. This suggests that the use of VS as an estimate of organic carbon content may not be accurate in predicting the proportion of leachable organic carbon. This may be affected by the types of sludges collected as shown by the WWTP_3 secondary sludge, which indicated high VS but a low DDOC. Together these measures may provide some greater insight into the overall organic carbon make-up of the sample (e.g. a high proportion of filterable organic carbon sources in the WWTP_3 secondary sludge).

Although this study was focussed primarily on the quantification of DDOC from various biosolids, the examination of trace metals in leachates indicates that DDOC could have an effect on relative fluxes of trace metals into the environment. The negative correlation between DDOC and Pb in this study suggests that biosolids with relatively low levels of leachable DOC may be more likely to allow transfer of some metals (such as Pb) into the environment. The study agrees with findings of Katsoyiannis and Samaras (2007) and Qi *et al.* (2011) noting the association between high total organic carbon content and lower metal leaching. High DDOC may indicate the presence of more binding and adsorption sites for Pb. However, this is contrary to results of Pinto and Al-Abed (2017) who observed low metal leaching in soils with low TOC concentrations, indicating that other features of sludge or biosolid matrices such as the presence of inorganic constituents may also be important to retention or leaching of metals.

7.6 Conclusions

This study has provided a quantification of DDOC from municipal wastewater treatment sludges and biosolids taken from a range of treatment plant sizes, processes and treatment stages, using a modified SPLP method. From the review of the literature, this is the first study of this type, measuring leachable DOC from biosolids using this method. The results provide data on a key characteristic of biosolids (DOC) that is of direct relevance to the transfer of organic micropollutants into the environment. The study also provides valuable information on the effect of wastewater treatment processes on the quantity of DDOC found in sludges. In particular the study has shown that nitrification can decrease DDOC in sludges recovered from secondary treatment processes. The effect of sludge treatment processes (AD and thermal treatment) was also observed to influence the quantity of DDOC in various biosolids, resulting in a reduction of DDOC in treated biosolids, with thermal treatment providing greater reduction than AD.

From the literature, quantifying the effect of treatment processes for individual compounds presents a complex and confusing picture. Process adaptations can have various effects on different types of compounds under differing conditions, making determination of the optimal treatment process difficult, and defining good reference compounds for assessing process efficiency difficult. More generic and environmentally relevant bulk-quantification methods that allow for comparison of biosolids are needed. The use of a comparable and repeatable methodology for estimating DDOC fluxes from sludges and biosolids can improve understanding of the impacts of land application of these materials. DOC movement into the environment can provide information on the transfer of both inorganic and organic micropollutants from wastewater treatment sludges and biosolids into the environment and determination of DDOC provides an initial step in improving understanding of the mobility of organic contaminants in sludges and biosolids. Further work is required to characterise DDOC, including the relative biodegradability and key properties of desorbed organic carbon to better assess risks of land application, or effectiveness of treatment processes. This is explored further in the chapters that follow. Areas of additional investigation not covered in this study could include toxicity characterisation of DDOC leachates, and research to better understand the relationship between DDOC and metal transport from various biosolid types.

CHAPTER 8 DETERMINATION OF PDDOC: BIODEGRADABILITY OF DOC DESORBED FROM BIOSOLIDS

8.1 Introduction

This chapter presents the experimental work carried out to assess the biodegradability of DDOC from the previous study. The persistence of organic compounds in the environment is one of the key characteristics used by environmental agencies in determining their potential for further surveillance and monitoring. It is the persistence of compounds that allow for continued exposure and bioaccumulation in susceptible receptor organisms. As presented in Chapter 3, biosolids can be a sink for a vast array of POPs, and one of the key risks associated with application of biosolids to land is the potential transfer of POPs into the environment. The literature suggests that more study is needed to characterise the nature and biodegradability of the soluble fraction of municipal wastewater treatment sludges applied to land, and to understand the fate of organic carbon in various treatment plant types and stages (Clouzot *et al.* 2012; Sozen *et al.* 2013). Techniques that allow for characterisation of the organic fraction of municipal wastewater treatment biosolids while overcoming the analytical barriers associated with determining absolute concentrations of specific compounds (e.g. matrix effects, sample pre-treatment, costs of analysis and skilled operators) may be useful to environmental regulators in estimating environmental risks.

Chapter 7 provided a quantification of the DDOC that may transfer from biosolids spread to land, using techniques used to simulate leaching in the environment. The concentration of DDOC represents a mixture of organic compounds including organic matter originating from human detritus, burst bacterial cells, and other natural sources of organic carbon alongside a range of organic contaminants including pharmaceuticals, personal care products and industrial chemicals that may have accumulated in the wastewater sludge matrix. To better characterise this mixture of organic carbon, this chapter presents the experimental work to assess the relative biodegradability of DDOC isolated in the previous study. The study will assess the rate of biodegradability over 28 days and quantify the relative quantity of persistent DDOC (PDDOC) for sludges and biosolids from each treatment plant and treatment stage.

8.2 Methodology

8.2.1 Theoretical background

As discussed in Chapter 4, risk assessment for chemicals often applies degradation models that estimate the rate of decline of compounds in the environment after discharge or application to land. This concept could also be applied to sludge and biosolid leachates. There has been limited study to characterise the relative biodegradability of the leachable organic fraction of biosolids, and limited study on the effect of wastewater treatment processes on the relative quantity of biodegradable and persistent organic fractions. Greater understanding of these key characteristics of biosolids could be important to future risk management of application of biosolids to land.

The procedure used to assess the quantity of PDDOC in the various wastewater treatment sludge and biosolid leachates was based on Organisation for Economic Development (OECD) Guideline for Testing of Chemicals 301, which is used to assess the relative biodegradability of organic compounds in an aerobic aqueous medium (Kowalczyk *et al.* 2014). Typically, this study is used for individual chemicals, and classifies those that do not degrade by 70% after 28 days as not readily degradable. In this study, a single compound is not being evaluated, but rather a group of unknown chemicals represented by DDOC, therefore the remaining percentage is a mixture of compounds that are poorly degraded and some that are readily degraded. The purpose of the aerobic biodegradability study based on the OECD method 301 therefore was to estimate the quantity of total PDDOC remaining and to compare this for sludges and biosolids from various treatment plants and following various treatment processes. The test results are used to provide an estimation of relative biodegradability of the compound mixture.

Following the aerobic experiment, a 10 day anaerobic screening test under mesophilic conditions was performed to assess whether the remaining DOC within samples would be further degraded under anaerobic conditions. The literature finds that some compounds are more readily degraded under anaerobic conditions (Rogers 1996; Carballa *et al.* 2007a,b; Barret *et al.* 2012), therefore this experiment was intended to identify if there is evidence that the resistant fraction remaining after the aerobic test, could potentially be further degraded. The anaerobic test followed the aerobic study rather than preceding it, given that biosolids spread to land will initially be exposed to aerobic conditions. Materials leaching from biosolids have the potential to migrate into waterways, or leach further into soils, where anaerobic conditions may dominate. Full anaerobic biodegradability studies following OECD guidelines are typically performed under mesophilic conditions (e.g. 35°C) and can last up to 60 days, with this extended time frame needed for an adaptation phase for the anaerobic microbial consortium. Suflita and Concannon (1995) demonstrated that using a pre-adapted microbial consortium can avoid the time lag associated with the adaptation period. Other biodegradability studies have demonstrated that a 10 day benchmark can be used as an indicator of anaerobic biodegradability, and for readily biodegradable compounds (e.g. glucose), 10 days is sufficient to result in near complete biodegradability under anaerobic conditions (Birch *et al.* 1989). While 10 days is not the usual length of test used to study anaerobic biodegradation, the use of an inoculum considered to be pre-adapted may be suitable and allow for a shorter test to be undertaken that can avoid an extended adaptation phase. For example, an inoculum from an anaerobic digester treating wastewater treatment sludges, may be considered pre-adapted to this type of substrate, and could be considered pre-adapted to the sludge leachates studied in this experiment..

8.2.2 Aerobic study

The test conditions specified in the OECD guideline include adding a known quantity of the sample solution into a mineral medium (feed water) that is inoculated with an appropriate microbial consortium and incubated under aerobic conditions in the dark or diffuse light at ambient temperature (e.g. 20°C).

8.2.2.1 Preparation of feed water

The feed water provides a pH buffer and micro-nutrients to sustain a health microbial consortium but no external sources of organic carbon, such that the only available organic carbon provided in the sample is DDOC. The feed water used in this study is similar to what is used in biological oxygen demand (BOD) studies and was produced in a 5 L batch starting with 5 L of ultrapure water (*Puracel* PURITE Select) aerated for a minimum of 2 h before use. 1 mL of each of the following nutrients and buffer components was pre-prepared in the Abertay Public Health Laboratory and added to 5 L of aerated ultrapure water:

- Phosphate buffer (pH 7.2): prepared by dissolving 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2 HPO_4 \cdot 7H_2O$, and 1.7g NH_4Cl in 0.7 L distilled water in a 1 L volumetric flask, then made up to 1 L with distilled water;
- Magnesium sulphate (1 $g \cdot L^{-1}$ stock solution): prepared by dissolving 22.5 g $MgSO_4 \cdot 7H_2O$ in 0.7 L distilled water in a 1 L volumetric flask, then made up to 1 L with distilled water;
- Calcium chloride (1 $g \cdot L^{-1}$ stock solution): prepared by dissolving 36.4 g $CaCl_2 . 2H_2O$ in 0.7 L distilled water in a 1 L volumetric flask, then made up to 1 L with distilled water;
- Ferric chloride (1 $g \cdot L^{-1}$ stock solution): prepared by dissolving 0.25 g $FeCl_3 \cdot 6H_2O$ in 0.7 L distilled water, with a few drops of HCl added to ensure stability, and made up to 1 L in a volumetric flask and stored in glass bottle.

The inoculum used in this study to provide the microbial consortium was, as recommended in the test method, a fresh final effluent from a local WWTP. In this case, the inoculum was supplied by WWTP_1.

8.2.2.2 Sample preparation

An aliquot of filtered leachate from the DDOC test was added by pipette to a clean, sterile 250 mL BOD sample bottle, with the exact volume added recorded for each sample. The quantity of sample added was based on the known starting concentration of the undiluted sample DDOC results to ensure adequate DDOC in the starting sample. The aerated feed water containing the microbial consortium, pH buffer and micro-nutrients was added to make each sample solution up to 250 mL. Sample bottles were covered with foil and placed in a dark incubator at 20°C until ready for subsampling (Figure 8-1).

Figure 8-1 Biodegradation test bottles

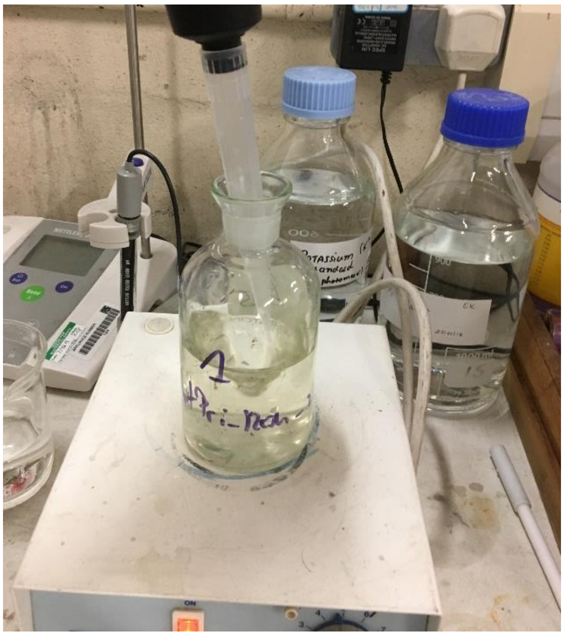


Figure 8-2 Subsampling for biodegradation tests

Subsampling occurred after 1, 2, 7, 16, and 28 days. For each subsampling event, sample bottles were removed from the incubator, and stirred before subsampling with a metallic stir bar (Figure 8-2). This was done to ensure a homogenous subsample was obtained and to reaerate the sample before returning to the incubator. An automatic pipette was used to extract a subsample, which was filtered directly through a 0.45 μm filter paper into a clean amber coloured centrifuge tube.

Sample bottles were then covered and returned to the incubator until the next subsampling event. The filtered subsamples were labelled with sampling time and date and placed in a refrigerator at 4 ± 2°C for analysis within 24 h, or in a freezer at -18 ± 2°C for analysis beyond 24 h. The DO concentration was measured (as described in Chapter 6) before final sample collection on day 28 and recorded in Appendix 8. Some of the remaining leachates were selected for use in a 10 day anaerobic biodegradation experiment, as detailed below.

8.2.2.3 Quality control

Triplicate samples of each sludge or biosolid leachate were used in the biodegradation experiments. Parallel inoculum and feed water blanks were used to control for additional contribution of DOC in the test matrix from the inoculum or minor sources of contamination. A readily biodegradable reference compound (potassium hydrogen phthalate, KHP) was used as a reagent spike. Spikes were made up using 25 mL of 1000 mg$\cdot$L^{-1} KHP (preparation described in Chapter 6) made up to 250 mL with feed water for a 100 mg$\cdot$L^{-1} KHP solution. An initial 25 mL aliquot was immediately sampled and recorded as the starting DDOC concentration (D0) for the spike. A blank sample containing feed water only was used to confirm the baseline level of DOC in the inoculum.

8.2.3 Anaerobic experiment

A subset of samples used in the aerobic experiment was selected for the anaerobic biodegradation batch test. This included triplicate samples of WWTP_1 biosolid (old), WWTP_1 primary sludge (May), WWTP_1 secondary sludge (May), WWTP_3 secondary sludge, WWTP_3 biosolid (BS) and the reference material. These samples were selected due to adequate sample volume, sufficiently high concentrations to allow for detectable changes in DOC, and diverse samples to allow for a comparison of sludges from nitrifying and non-nitrifying plants and biosolids of three types. One blank and one spiked sample were used per batch.

The anaerobic tests immediately followed the aerobic tests. Due to small remaining sample volumes, small batch bottles were used (e.g. 100 mL). Remaining sample (e.g. 100 mL) from the aerobic experiment was transferred directly to clean 100 mL amber glass bottles. Each sample was found to have starting DO concentration > 6.0 mg$\cdot$L^{-1} at the end of the biodegradation study therefore a method to remove DO from leachates was identified.

In this phase of the study, a process that allowed for immediate deoxygenation of samples to halt aerobic activity, without affecting DOC concentration was used based on methods described by Rollie *et al.* (1987). Given the need to deoxygenate and halt aerobic activity as quickly as possible, a sodium sulphite method was used. Sodium sulphite quickly and effectively reacts with oxygen in solution to form sodium sulphate, consuming oxygen in the process creating anaerobic conditions as described in Equation 4.

$$2\,Na_2SO_3 + O_2 \longrightarrow 2\,Na_2SO_4$$

Equation 4 Reaction illustrating the consumption of oxygen by sodium sulphite to form sodium sulphate

Based on measured DO concentrations in the samples, an appropriate dose of sodium sulphite was added to deoxygenate the samples. This amounted to approximately 3 mL of 10 $g{\cdot}L^{-1}$ Na_2SO_3 (hydrate) added to each sample. This was found to reduce DO in all samples to < 0.5 $mg{\cdot}L^{-1}$ at the beginning of the experiment.

As previously indicated, the use of an inoculum from an anaerobic digester at a WWPT was considered to be the most likely to be pre-adapted to the leachates assessed in this study. This was an area of uncertainty in the experiment; however, the anaerobic test was carried out primarily to identify if there was any indication that additional biodegradable material remained that was not digestible by the aerobic microbial consortium. 1 mL of anaerobic inoculum (fresh digestate obtained from WWTP_1) was added to each sample to provide the anaerobic microbial consortium. A dilution factor of 1.04 was applied to all samples to account for addition of Na_2SO_3 and AD inoculum. Samples were sealed with rubber stoppers and wrapped tightly with clingfilm to prevent ingress of O_2 into the samples. Samples were placed in a dark incubator at 37°C for 10 days (Figure 8-3). At the end of the incubation period, the DO concentration was measured for each sample, with most samples found to be below 0.5 $mg{\cdot}L^{-1}$ DO, but four samples (WWTP_1 primary, replicates 1 and 2; WWTP_2 secondary, replicate 2 and WWTP_3 biosolid, replicate 1), and the blank were observed to exceed this. A comparison of the three replicates for each of these samples identified no evidence of differences in DOC between the samples with final DO > 0.5 $mg{\cdot}L^{-1}$ and those with DO < 0.5 $mg{\cdot}L^{-1}$. The batch blank was found to have the highest DO at 3.19 $mg{\cdot}L^{-1}$ indicating that anaerobic conditions were not maintained. It is possible that some anaerobes would not tolerate this level of oxygen and may die off. The final DO results are presented in Appendix 8.

Figure 8-3 Anaerobic biodegradation test sample bottles

Following measurement of DO, each sample was again filtered through a 0.45 µm filter paper into a clean, amber coloured centrifuge tube, and placed in a refrigerator at 4 ± 2°C for analysis within 24 h, or a freezer at -18 ± 2°C for analysis taking place later.

8.2.4 Determination of PDDOC

DOC was determined using a TOC analyser (*OI Analytical* 1010 as described in Chapter 6). Dilutions, where necessary, were carried out using ultrapure water (*Puracel* PURITE Select). The DDOC results from the previous experiment were recorded as the day 0 (D0) concentration of DOC. The DOC was measured on day 1, 2, 7, 16 and 28 (D1-D28) and relative % biodegradation was calculated. Percentage degradation (D_t) was determined after each sub-sampling event and DOC analysis using Equation 5.

$$D_t = \left[1 - \frac{C_t - C_{bl(t)}}{C_o - C_{bl(o)}}\right] x\ 100$$

Where:

D_t = % degradation at time *t*;

C_o = mean concentration of DOC in the sample ($mg \cdot L^{-1}$ DOC) at time 0;

C_t = mean concentration of DOC in the sample ($mg \cdot L^{-1}$ DOC) at time *t*;

$C_{bl(o)}$ = mean concentration of DOC in the blank ($mg \cdot L^{-1}$ DOC) at time 0;

$C_{bl(t)}$ = mean concentration of DOC in the blank ($mg \cdot L^{-1}$ DOC) at time *t*;

Equation 5 Calculation of relative % biodegradation of DOC

The persistent DDOC (PDDOC) was then recorded as the concentration remaining on day 28.

8.2.5 Limitations of the method

Although the OECD biodegradability test method is used widely for chemical screening and provides the foundation for regulation on chemical persistence, there are some limitations in the test (Kowalczyk *et al.* 2014). This includes the choice of inoculum, which is stated to be flexible within the test guidelines with options to use activated sludge, municipal effluent, river water or soil. Different source inoculum may have different communities of microbial degraders, and the rate of degradation may differ if an adaptation phase is required. In this study the inoculum was a municipal effluent from one of the treatment plants being tested. This type of inoculum is already adapted to the municipal effluent environment, and hence the degraders present are less likely to require an adaptation phase as compared to inoculum sourced elsewhere.

Another limitation is the fixed laboratory conditions of pH and temperature, which may be less likely to represent fluctuating environmental conditions. Field tests may provide different results than those obtained under laboratory conditions. In the context of Scotland, where maximum average monthly temperatures during summer reach between 15-17°C, the warmer laboratory conditions (20°C) may favour a slightly higher rate of degradation and may slightly over-estimate the rate of biodegradability. Similarly, the laboratory test conditions may not be an adequate representation of conditions found in a tropical setting where ambient temperatures may be much higher. Maximum growth rates of microbial degraders are temperature dependent, and an increase of 10°C can lead to a doubling of microbial activity (Clara *et al.* 2005), therefore temperature may be an important factor in predicting biodegradation rate of readily biodegradable material in the field. Temperature may however have little impact on the degradation of very persistent compounds, but this could be an area for further study. Finally, there is no certainty that all the DOC remaining after the 28 day limit specified in the method will not biodegrade over time. This time limit is somewhat arbitrary in the literature; however, it is the standard timeframe used in industry and regulatory screening, and there is no evidence that chemicals with slower degradation (e.g. 60 days rather than 28 days) necessarily pose a greater risk to human health and the environment (Kowalczyk *et al.* 2014).

Finally, there may have been some limitations in the anaerobic test. One of the limitations is the trace amount of DO present in some sample vessels at the beginning of the experiment and the ingress of DO throughout the experiment for a small number of samples, which may have affected the viability of some anaerobic bacteria. Second, the 10 day time frame may have been insufficient for some bacteria to acclimatise to the conditions in the test vessel. Although it was hoped that the use of a pre-adapted microbial consortium would allow for a reduced adaptation period, there did not appear to be additional degradation of organic carbon over the 10 day experiment. Further study would be needed to assess the effect of a longer adaptation phase on additional degradation under anaerobic conditions, however there may have been other factors affecting the viability of the anaerobes, unrelated to the adaptation period. Third, the use of rubber stoppers to seal the bottles may have resulted in leaching of DOC into the samples causing a spike in DOC results following the experiment. Small sample vessels of just 100 mL were used in this experiment, so the relative contribution of leaching from the stoppers could have been significant. Finally, the levels of nutrients and degradable organic carbon remaining in the sample may have been insufficient to sustain the microbial population. As demonstrated in the literature, degradation of persistent materials may be as a result of co-metabolism with other more readily biodegradable material (Delgadillo-Mirquez *et al.* 2011). In their absence, some bacteria may have been unable to survive. In addition, the concentration of non-degradable substances in the sample solutions could also have contained a relatively higher proportion of resistant and toxic compounds, hence affecting microbe viability.

8.3 Results

8.3.1 Persistent DOC (PDDOC)

The full results of the 28 day aerobic biodegradation study are presented in Appendix 8. For all samples, the concentration of DOC was reduced after the 28 day aerobic biodegradation test but little or no reduction (some increases) were observed after the anaerobic stage. Table 8-1 presents the mean PDDOC ($mg{\cdot}kg^{-1}$) results for the 28 day aerobic (D28) and 10 day anaerobic tests (D38) (where applicable) alongside overall biodegradation (%) at both end points with Figure 8-4 providing a graphical representation of these values.

Table 8-1 Mean PDDOC (concentration and % biodegradation) after 28 day aerobic biodegradation test (D28), and 10 day anaerobic biodegradation screening test (D38)

Sample	**PDDOC D28 (mg·kg^{-1})**	**D28 (% degraded)**	**PDDOC D38 (mg·kg^{-1})**	**D38 (% degraded)**
1_BS(Apr)	7,547 (1,072)	68%		
1_BS(Old)	7,500 (633)	73%	7,589 (875)	73%
1_Pri(Apr)	5,322 (426)	75%		
1_Sec(Apr)	4,520 (916)	80%		
1_Pri(May)	4,657 (15)	79%	5,401 (343)	76%
1_Sec(May)	4,854 (338)	86%	5,007 (482)	85%
2_Pri	5,822 (907)	79%		
2_Sec	5,740 (230)	75%		
3_Sec	4,574 (330)	61%	4,871 (871)	59%
3_BS	6,122 (160)	80%	8,752 (54)	71%
4_Sec	4,096 (561)	80%		
Ref	5,600 (484)	65%	5,021 (261)	61%

(Standard deviation shown in brackets)
BS = biosolid, pri = primary sludge, sec = secondary sludge, Ref = reference biosolid

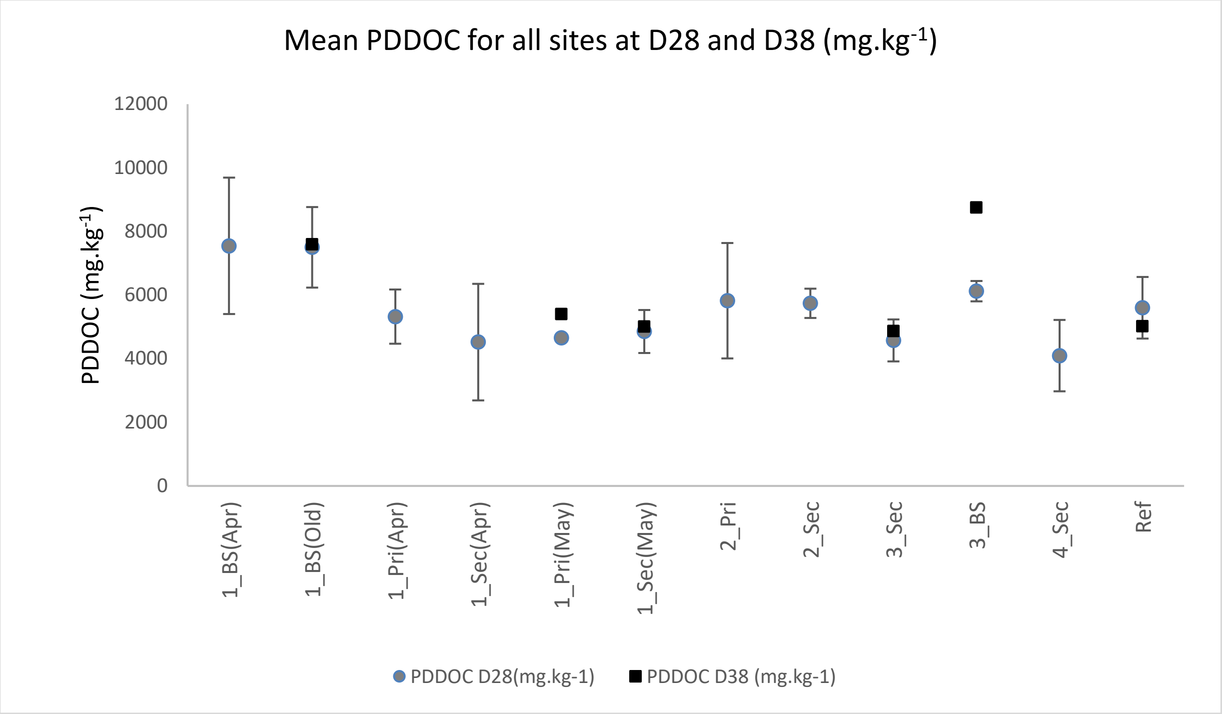


Figure 8-4 Mean PDDOC (mg·kg^{-1}) remaining after the 28 day aerobic (D28) and 10 day anaerobic tests (D38) *(error bars representing 2 x SD)*

This study found that between 61-85% of the desorbed organic carbon in the samples tested was biodegradable under the 28 day aerobic test conditions. The biosolids from WWTP_1 had the highest concentration of PDDOC remaining, with similar results obtained for biosolids collected in both sampling events for this site. Biosolids from WWTP_3 had the second highest PDDOC but also one of the highest percentages of readily degradable DDOC.

The results of the 10 day anaerobic screening did not show additional significant degradation, and most results showed higher concentrations of DOC than the preceding experiment. This may be due to contamination during sample handling and preparation and could include leaching from the rubber stoppers used to seal the batch bottles. However, correction for sample blanks with results corrected for contribution of the inoculum to DOC concentrations found that results were still higher than the starting day 28 concentration. An additional explanation for the increased results could be due to conditions in the sample vessel that did not favour cell survival and metabolism, resulting in microbial cell lysis and release of intracellular contents into the dissolved fraction.

8.3.2 Rate of Biodegradation

The biodegradation curve for all samples is shown in Figure 8-5. All samples followed a similar pattern of degradation with rapid degradation in the first two days. Three samples had a mean negative degradation rate at day 1 (D1). This could be explained by sample contamination; however, all samples were blank corrected for their respective batch blanks, which had minimal evidence of contamination (e.g. < 1 % of the lowest sample concentration). Another explanation is that the matrix in the biodegradation samples (leachates plus feed water) differs from the matrix in the DDOC samples, which was used as the starting day 0 (D0) benchmark. This may have resulted in analytical differences due to interferences in the feed water. The result may also be related to unfavourable conditions for the microbes present leading to cell death and lysis, releasing dissolved materials into the samples.

For some samples, there was a small decrease in biodegradation rate at day 16 (D16). This appears to only be associated with Batch 2 samples, suggesting analyst error, contamination or instrument interference on the day that batch 2 samples were sampled, filtered and analysed. Quality control blanks for day 16 did show higher DOC results than previous days, but this trend was found for both batches, therefore it may be more indicative of instrumental error. Results for day 28 resume the initial trajectory of the biodegradation curves. As the results for day 16 were only indicative for the purpose of mapping the trajectory of biodegradation, they do not affect the calculated PDDOC results.

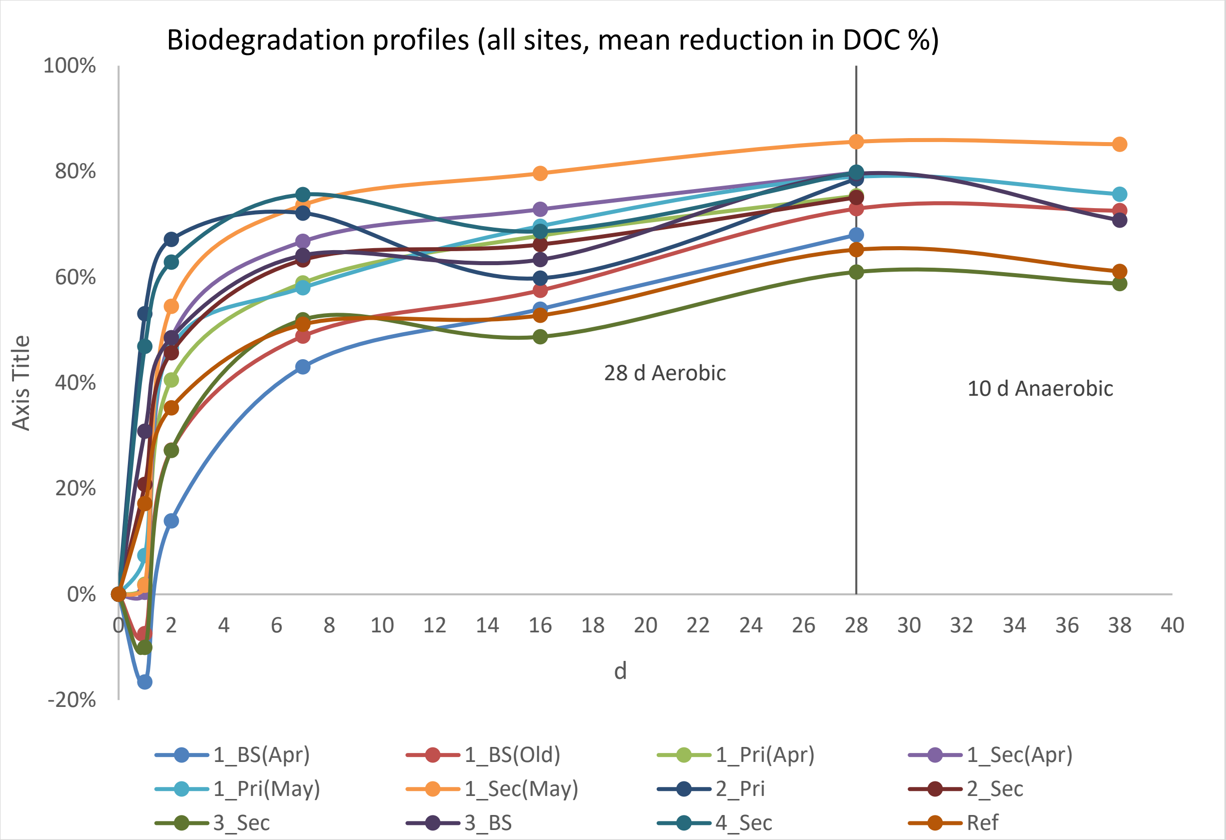


Figure 8-5 Biodegradation curve for all samples showing mean DOC reduction (%) by time (d)

A comparison of biodegradation curves for primary sludges is presented in Figure 8-6. The curves for primary sludges collected in April and May from WWTP_1 demonstrate a similar rate of degradation although final concentration was slightly higher for the April samples compared to the May. The similar biodegradation curves suggest the samples have similar ratios of readily biodegradable to non-biodegradable compounds. The primary sludge from WWTP_2 differs slightly, particularly at day 16, suggesting an anomaly in the dataset at day 16 as mentioned above. This aside, the sludge has a steeper degradation curve in the first two days, which levels off reaching a similar degradation rate at day 28. This suggests initially more readily biodegradable material (as a percentage of total DDOC) in WWTP_2 primary sludge compared to WWTP_1 primary sludge. This sludge was obtained from the smallest of the treatment plants, serving a small village and selection of commercial premises, compared to WWTP_1, which was the largest of the treatment with a much more diverse catchment including municipal, commercial, industrial, and hospital inputs.

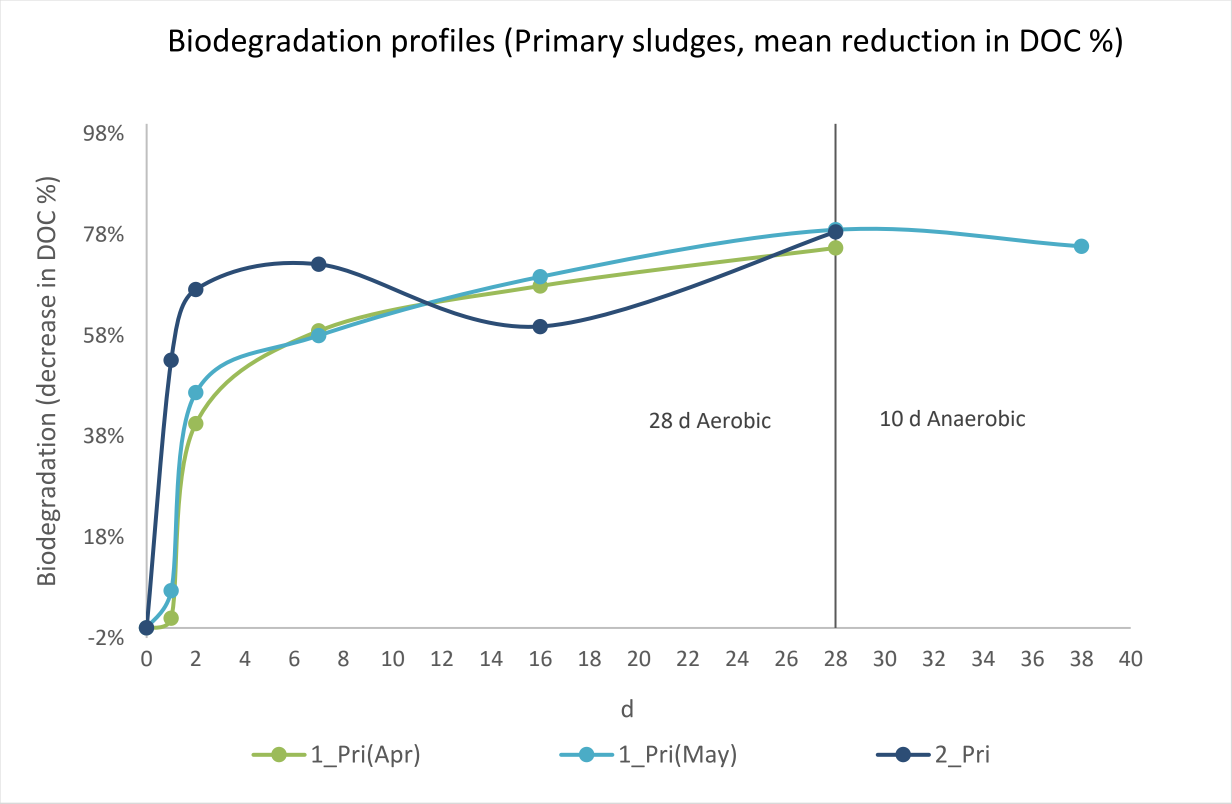


Figure 8-6 Biodegradation curve for Primary sludges showing mean DOC reduction (%) by time (d)

A comparison of biodegradation curves for secondary sludges from all four treatment plants is presented in Figure 8-7. The plants differ in their size and catchment characteristics, but also operational factors such as HRT and nitrification in the secondary treatment stages. All sites have a rapid two-day increase in degradation rate that shows signs of slowing at day 7 (D7). The key difference is the level of relative biodegradation based on the DDOC content. As expected, WWTP_3, a nitrifying plant with the longest retention time, shows a lower overall rate of biodegradation, followed by the other nitrifying plant (WWTP_2) and the part-nitrifying plant (WWTP_4). This is likely due to a greater proportion of biodegradable organic compounds already consumed within the plants with extended aeration allowing for nitrification processes to occur. In contrast, secondary sludges from the non-nitrifying plant demonstrate a higher relative biodegradation rate. The final day 28 PDDOC concentrations however at WWTP_1 are similar to WWTP_3 (4,520 and 4,854 mg·kg^{-1} for WWTP_1 April and May respectively compared to 4,574 mg·kg^{-1} for WWTP_3). This suggests that although overall biodegradation rates are different, the quantity of persistent material is similar.

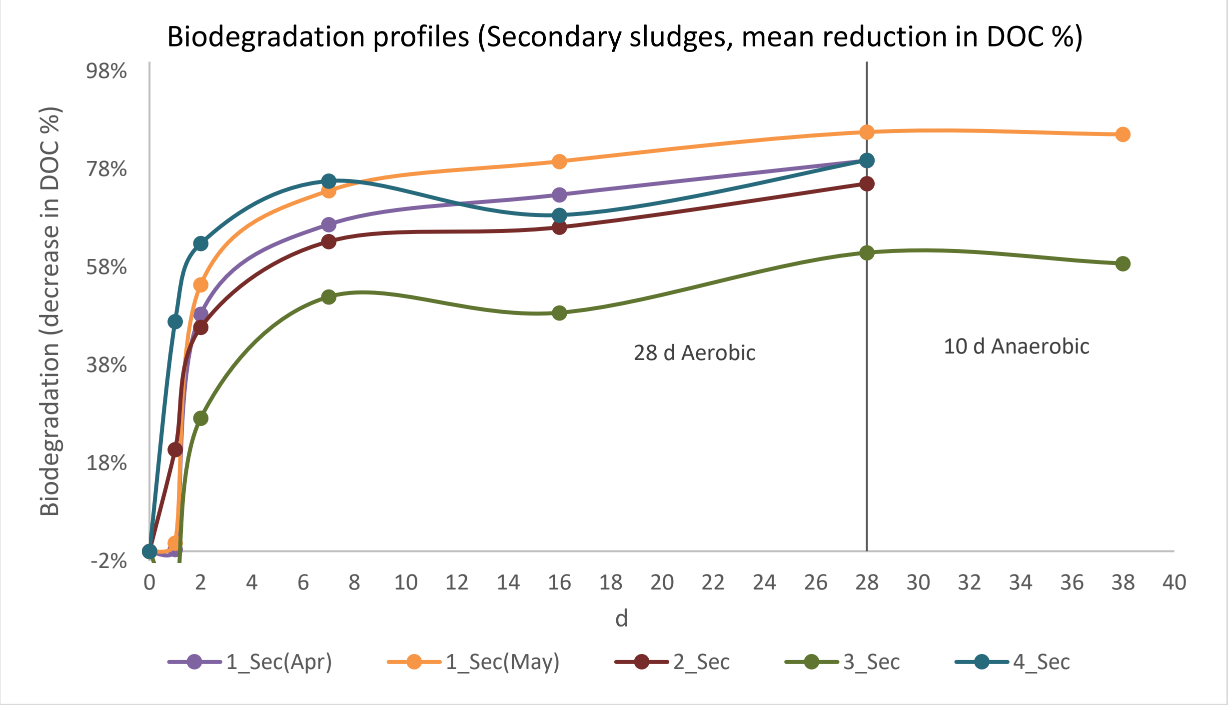


Figure 8-7 Biodegradation curve for secondary sludges showing mean DOC reduction (%) by time (d)

A comparison of biodegradation curves for biosolids is presented in Figure 8-8. Again, all samples show a rapid rate of biodegradation in the first two days, which then slows, with a more gradual reduction in DOC to day 28, and no additional degradation is observed in the anaerobic test. The biosolids from WWTP_3 represent treated sludge that undergoes only thickening with a polymer and dewatering before the sludge cake is transported to landfill. In contrast the biosolids from WWTP_1 are treated by dewatering, followed by AD and drying. The Reference material was treated by thermal treatment. The profiles for the biosolids appear to be much less of an inverted L-shape as seen for the other sludge types. Although the rapid increase in the first two days is similar, the slightly slower rate of degradation that follows may be indicative of more recalcitrant and hard to remove compounds remaining in the samples. The additional treatments these samples have undergone in contrast to the sludges obtained directly from the active treatment systems implies that a greater proportion of degradable organic carbon would already have been removed, leaving more recalcitrant substances behind.

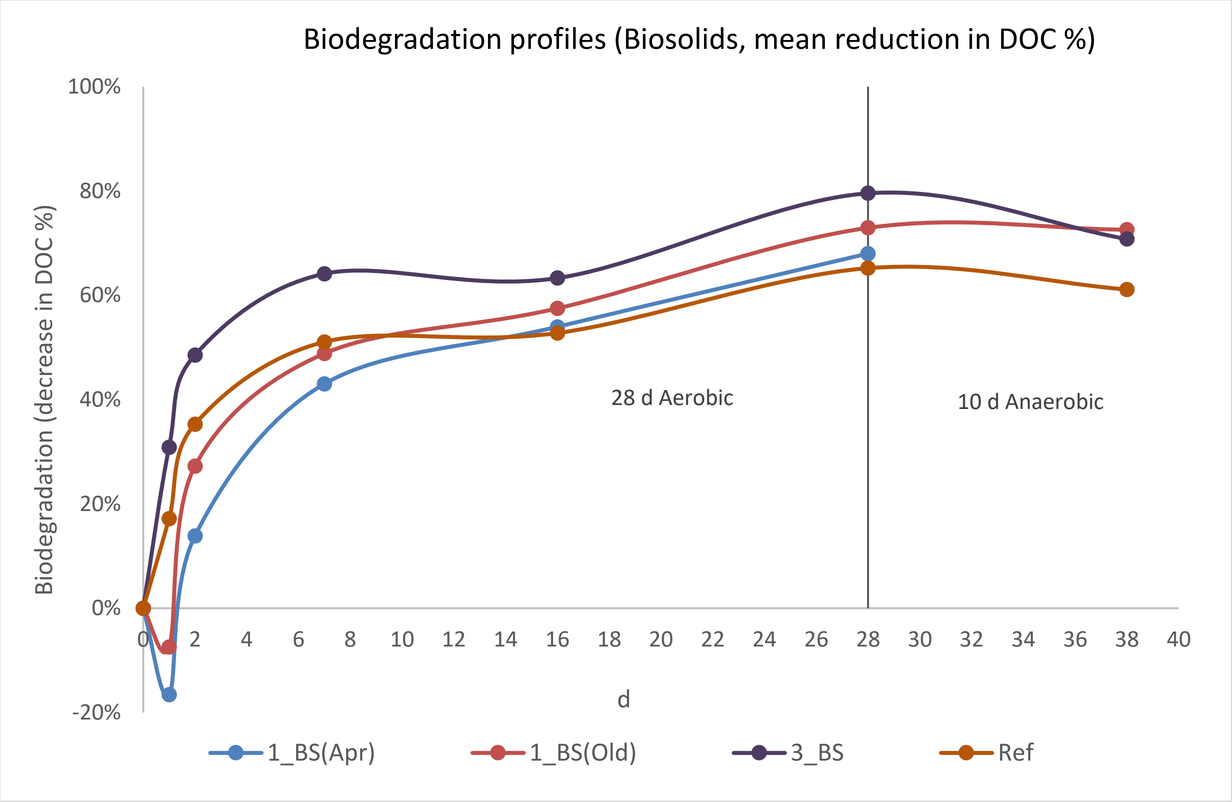


Figure 8-8 Biodegradation curve for Biosolids showing mean DOC reduction (%) by time (d)

8.3.3 Statistical analysis and comparison of PDDOC

Final PDDOC concentrations ranged from 4,096 mg·kg^{-1} (SD 561) to 7,547 mg·kg^{-1} (SD 1,072) across all sludge and biosolid samples. This is a narrow range of values compared to differences in DDOC observed in Chapter 7. Variation was observed in the percent biodegradation of DDOC from biosolid leachates, and the remaining PDDOC across sludge and biosolid types. A statistical comparison of mean PDDOC results was performed to test for any significant differences between the groups as presented in Table 8-2. Results were analysed using SPSS. Across the category of WWTP, all comparison tests (ANOVA, Non-parametric and multiple comparisons) found $p > 0.05$, suggesting that the null hypothesis (H_0: *there is no difference between treatment groups*) should be retained. In contrast, across the categories of process stage (Primary, Secondary or Biosolids), $p < 0.05$ for both ANOVA and non-parametric tests suggesting a difference exists between PDDOC from various process stages. The multiple comparison tests indicated that there was no significant difference between total PDDOC concentration in primary and secondary sludge samples, but there was a difference ($p < 0.05$) between primary sludge and biosolids and secondary sludge and biosolids. Finally, in the categories of nitrification, a comparison of secondary sludges found a significant difference between nitrifying, non-nitrifying and part-nitrifying plants (using both the ANOVA and non-parametric tests). The multiple comparison tests found that there was no difference between non-nitrifying and part-nitrifying sites, however there was a significant difference between nitrifying and both non-nitrifying and part-nitrifying sites.

Table 8-2 Summary of statistical comparisons of PDDOC results across WWTP, Process stages and level of nitrification

Variable	Significance (ANOVA)	Non-parametric tests (Kruskal-Wallis)	Multiple Comparison tests
WWTP	$p = 0.227$ (Retain H_0)	$p = 0.200$ (Retain H_0)	All comparisons $p > 0.05$
Process stage	$p = 0.000$ (Reject H_0)	$p = 0.001$ (Reject H_0)	Primary v Secondary $p > 0.05$ (0.420) Primary v Biosolids $p = 0.009$ Secondary v Biosolids $p = 0.000$
Nitrification (secondary sludges only)	$p < 0.05$ (Independent samples t-test) (Reject H_0)	$p < 0.05$ (0.032) across categories of nitrification (Reject H_0)	Nitrifying v Non-Nitrifying ($p = 0.01$, equal variances not assumed) Non-Nitrifying v Part Nitrifying ($p=0.31$, equal variances assumed/not assumed) Nitrifying v Part-Nitrifying ($p = 0.001$, equal variances not assumed)

8.4 Discussion

8.4.1 Biodegradation method

The results of the study indicate that the 28 day aerobic biodegradation test is a good predictor of relative biodegradability of DDOC for all the biosolids tested. The results after 28 days are relatively stable suggesting that maximum biodegradation has been achieved within this time frame. Most samples were found to reach near maximum biodegradation rate by day 7, after which there was little change in additional removal. This indicates that a test protocol with a reduced biodegradation time of 7 days may be a good predictor of the 28 day test. However, general characterisation of the biodegradation pathway for organic carbon content is complicated by the wide variation in removal efficiencies of various compounds (Onesios *et al.* 2009). Urase and Kikuta (2005) found that the lower the initial TOC concentrations in activated sludge processes, the higher the degradation rates of pharmaceuticals in municipal wastewater, and microbes will more rapidly degrade resistant compounds in the absence of easily biodegradable substrates. Further study over a wider range of sludge types from a wider range of treatment plants may be useful in refining a biodegradation test protocol appropriate for biosolids.

The results of the anaerobic study provided no additional evidence of biodegradability of remaining PDDOC under anaerobic conditions. There are several areas of uncertainty that have been discussed including potential sources of contamination, and unsuitable conditions for the anaerobic microbial consortium that may have resulted in the increased DOC readings in some samples.

8.4.2 Rate of biodegradation

All biodegradation curves showed an initial two-day rapid decline in biodegradable DOC followed by a slowing to day 7 and a plateau to day 28 (Figure 8-5). The biodegradability curves indicate first order kinetics for the first stage of degradation. For readily biodegradable materials degraded in this stage, the rate of degradation is only limited by the rate of metabolism of the degraders, and degraders will choose easy to degrade materials first. As readily biodegradable materials become depleted, rate of degradation declines and levels off, even though DOC has not been depleted. This provides a good indication that the remaining DOC is harder to degrade by the microbial consortium that is present. As shown by Purdy and Cheplick (2014), the rate of decline of more persistent compounds may not follow first order models (e.g. as shown with the pesticide chlorpyrifos in soil) and nonlinear soil dissipation kinetics are observed for persistent compounds.

For some organic compounds, biodegradation rates are found to plateau, but this can be compound specific and may relate to the presence of other sources of organic carbon, or over time the biotransformation of some compounds may release more readily biodegradable sources (Blair *et al.* 2015). Literature finds that some compounds have multiple transformation by-products. For example, ciprofloxacin has over 60 transformation by-products and enhanced treatment processes may be needed to achieve more complete degradation (Haddad *et al.* 2015). However, this study suggests that enhanced treatment may need to go beyond conventional approaches of enhanced biological treatment or thermal treatment to achieve reduction of the more persistent POPs.

8.4.3 Persistent organic carbon

Total PDDOC was found to range from about 4,000 to 7,500 mg·kg^{-1} across various sludge types. Primary and secondary sludges had a similar range of values (4,657-5,800 and 4,096-5,740 respectively). The range for the biosolids was higher (5,600-7,547 mg·kg^{-1}).

Leachate from sludges and biosolids that had undergone processes to reduce organic carbon during the wastewater treatment process (e.g. secondary extended aeration in nitrifying plants and additional treatment of biosolids) show a lower level of overall biodegradability. In addition, these sites (WWTP_1 biosolids and WWTP_3 nitrifying secondary treatment plant sludge) show the highest concentrations of PDDOC. This aligns with the predicted outcome that a greater proportion of the DOC in these leachates would be persistent, given that more biodegradable organic carbon removal occurred during the wastewater treatment process. This also agrees with results of Martin *et al.* (2012) who found that total concentrations of less biodegradable compounds increased from primary sludge to secondary sludges. Despite the additional treatment that the biosolids have undergone, there is a greater concentration of persistent organic carbon in leachates for these samples. The results suggest that additional treatments to biosolids to reduce the overall organic carbon load in the treatment plants result in either concentration of less biodegradable materials, or mobility of a higher proportion of less biodegradable material. Some of this material may include persistent parent compounds or persistent transformation by-products from partially metabolised substances. In addition, as described in the literature review, the presence of organic matter can influence sorption of POPs. Additional sludge treatments, such as thermal treatment, may reduce the quantity of organic matter in the biosolid matrix, resulting in desorption of POPs. This may increase the relative proportion of persistent compounds desorbed under leaching conditions.

The differences observed for secondary sludges may not be based solely on the nitrification process. Although the secondary sludge with the highest PDDOC is from a nitrifying plant, the second highest is from a non-nitrifying plant, and the lowest is from a part-nitrifying plant. These differences could be related to catchment but may also be linked to SRT. As noted, there is a difference in mean PDDOC for the two nitrifying plants. The one with the longest SRT (WWTP_2) has the highest PDDOC, compared to the results from WWTP_3, which is lower, and has a reduced SRT. An increased SRT has been found to result, in general, in a higher rate of removal of POPs from effluent, which could be due to additional sorption to solids (Jelic *et al*. 2011). This may be due to increased time for sorption to occur (Kinney *et al*. 2006).

Abegglen *et al.* (2009) found that nitrifying WWTP with SRT of ≥ 8 days have higher elimination rates for micropollutants compared to plants with no nitrification. However, they also find that high HRT and SRT have little effect on very persistent pharmaceuticals, which are not removed in either large or small WWTP (e.g. diatrizoate). The literature has shown that biodegradation processes will not degrade recalcitrant compounds even with extended HRT or SRT. For example, the anti-epileptic drug carbamazepine has been shown to resist degradation regardless of changes to SRT or microbial activity (Maeng *et al.* 2013). Other pre-treatments, not used on sludges studied in this work, such as physico-chemical methods, may be more efficient than biological or heat treatments. For example, photo-catalytic oxidation has been found to be effective for removal of compounds such as BPA, and other methods such as ultrasound can cause reactive radicals to be produced that can aid decomposition of POPs (Mohapatra *et al.* 2010). Removal of the persistent fraction of biosolids may therefore need further investigation for regulators to be able to recommend suitable treatment options, beyond adjustments to conventional WWTP operational parameters.

8.5 Conclusions

The results of this study have identified that the PDDOC approach may be a useful tool in comparing mobile and persistent fractions for sludge and biosolid leachates.

All leachates from all sludge types tested were found to contain an appreciable concentration of persistent organic carbon. This study finds that sludges from plants that have undergone additional organic reduction, or biosolids that result from sludge treatment may have higher concentrations of persistent compounds in the desorbable fraction. This result has implications for current practice in the management of biosolids. Thermal treatment or AD for reduction of organic content may be useful for reducing biodegradable content but may result in increased mobility or concentration of persistent compounds.

There is potential for the approach used in this study to be applied to risk assessment of application of biosolids to land. It may also be a useful tool in the assessment of effectiveness of post-WWTP treatment options for biosolids. Further characterisation of the leachates to identify the composition could help to establish with greater confidence the linkages between PDDOC measures and persistent pollutant content. Additional characterisation could include identification of individual pollutants and quantification of concentrations, toxicity testing on leachates, or optical property analysis to identify key compound types and groups that may be present in leachates. The latter approach will be used in the following experiment to provide further characterisation of PDDOC.

CHAPTER 9 QUALITATIVE ANALYSIS OF BIOSOLIDS AND LEACHATES BY UV AND FTIR

9.1 Introduction

The previous chapters have provided an estimate of the quantity of DDOC and PDDOC for various biosolids. Further characterisation of the leachates could assist in understanding the types of compounds in various leachates. This in turn would provide an indication of the types of compounds that are likely to leach from biosolids applied to land and persist in the environment, and the influence of wastewater treatment processes on the resulting leachates. While a compound by compound examination of each solution using modern analytical techniques could be applied, this would be both costly and time consuming, and be limited to pre-selection of compounds predicted to be present. A more generic qualitative analysis, however, could provide an indication of the types of compounds present and help to better understand degradation processes. The use of techniques such as UV and FTIR could be useful for both examination of dominant functional groups in dried solids, DDOC and PDDOC, to compare how the DOC profile differs across sample types and treatments. This chapter provides the results of a study to characterise the biosolids and leachates using techniques of UV-Vis wavelength scanning (for leachates) and Fourier transform infrared (FTIR) analysis (for dry solids and leachates).

9.2 Methods

9.2.1 Theoretical background

One of the key challenges to environmental regulation of organic compounds in substances such as biosolids applied to land is the large number of potential compounds and mixtures present, including a potentially large number of unknown substances. Generic tools such as UV-Vis wavelength scanning and FTIR analysis, as introduced in Chapter 4 have been used widely to help characterise compound mixtures and examine decomposition or degradation processes.

9.2.1.1 UV-Vis

UV-Vis wavelength scanning has been used to characterise dissolved organic matter and dominant compounds in fresh and marine water systems, wastewater and landfill leachate (Saadi *et al.* 2006; Antilen *et al.* 2014; Li *et al.* 2014; Knapik *et al.* 2015; Mesquita *et al.* 2017). The underpinning principle for the technique is that light in the UV to visible wavelength range (e.g. 200-700 nm) passed through a liquid sample will be absorbed by various compounds and structures at characteristic wavelengths. The individual components of a compound that absorb UV and/or visible wavelengths are called chromophores. The wavelength (λ) or absorbance can be associated with various chromophores, and the degree of absorbance can be associated with the compound concentration. UV-Vis has also been used to develop absorbance ratios (λ_1:λ_2) to quantify degradation of parent compounds into metabolites by examining changes in ratios. Specific UV absorbances (SUVA) have also been used to characterise DOC (Weishaar *et al.* 2003; Hansen *et al.* 2016). For example, $SUVA_{254}$, which is a ratio of absorbance at 254 nm and the DOC concentration, can provide an indication of aromaticity. Aromatic compounds such as benzene and polycyclic aromatic hydrocarbons are of concern due to their persistence and potential toxicity and carcinogenicity (Schramm and Kettrup 2004). Knapik *et al.* (2015) used UV-Vis to measure changes in absorbance at 254 nm to evaluate the biodegradation of anthropogenic (wastewater derived) organic matter in river waters and Li *et al.* (2014) used UV-Vis spectra to characterise changes in dissolved organic matter (DOM) during municipal waste composting. The technique has been found to offer ease of use and can improve understanding of DOC and characterising trace organic pollutants using single absorption wavelengths, wavelength scans and absorption ratios to infer properties such as molecular weight, aromaticity, and the sources of DOM (Li and Hur 2017).

9.2.1.2 FTIR

As discussed in Chapter 4, FTIR has been used to examine dominant functional groups in various leachates and digestate products from various source materials, (Provenzano *et al.* 2011; Soong *et al.* 2014; Reinhart and Bolyard 2015). In FTIR analysis, infrared radiation is directed towards a test sample, with radiation either being more absorbed or more transmitted through the sample. A detector measures the transmitted radiation and generates a spectrum that can be interpreted to identify characteristic spectra for various functional groups. Many individual compounds have fingerprint spectra that can be used to confirm single compound purity, or to identify the key dominant functional groups in a compound mixture and therefore FTIR is often used in pharmaceutical analysis to identify impurities in a product or to examine dissolved organic matter in a range of matrices including landfill leachates for the main functional groups (Soong *et al.* 2014). FTIR has also been used in a few cases to characterise municipal wastewater treatment sludges (de Oliveira Silva *et al.* 2014; Singh *et al.* 2017).

9.2.2 Analytical Methods

9.2.2.1 UV-Vis

Sample preparations from DDOC (Chapter 7) and PDDOC (Chapter 8) experiments were selected for wavelength scans. Replicate leachates were analysed where adequate volume remained following previous testing rounds. Inadequate sample volumes from WWTP_2 leachates were available for scanning. Where required, samples were diluted using ultrapure water (see Chapter 6) and finale absorbances adjusted for dilution factors. Wavelength scans were performed using a DR 5000 spectrophotometer (*Hach-Lange*). The wavelength scan range was 190 – 650 nm, with 1 nm resolution and an absorbance range of -0.300 to 3.500 absorbance units per cm. A 1-inch glass sample cell was used for each scan. A baseline wavelength scan using ultrapure water was performed at the beginning of each round of analysis as per instrument instructions. Wavelength scans of process blanks were also performed to identify potential sources of contamination and a reference scan of 100 $mg{\cdot}L^{-1}$ potassium hydrogen phthalate (KHP) was run (Figures 12-7, 12-8, 12-9, Appendix 9).

Scans of samples were performed on day 0, day 28 and day 38 (AD) leachates, with results exported to Microsoft Excel for graphical analysis and calculations. Plots of absorbance versus wavelength (nm) were overlaid for comparison of replicates of day 0, day 28 and day 38 for various sample types and sites respectively. Absorbance ratios were calculated using mean absorbances at $\lambda_1{:}\lambda_2$. $SUVA_{254}$ (reported as $L{\cdot}(mg{\cdot}m)^{-1}$) was calculated using mean absorbance (A) at λ254 (cm^{-1}) and mean DOC (μ_{DOC}) concentrations in $mg{\cdot}L^{-1}$ as shown in Equation 6.

$$\mathrm{SUVA254} = 100 \times \frac{\bar{\mathrm{A}}254}{\mu\mathrm{DOC}}$$

Equation 6. Calculation of specific UV absorbance at 254 nm (SUVA$_{254}$)

9.2.2.2 FTIR

Samples from DDOC and PDDOC experiments were selected for FTIR analysis where adequate sample volume remained following previous test rounds. The dry solids used as starting materials in the DDOC experiments were also analysed using FTIR. These samples were ground to a powder using a mortar and pestle before analysis. Analysis was carried out using a Nicolet Is5 Spectrophotometer (*Thermo Fisher*) with OMNIC peak analysis software and equipped with the ATR iD5 crystal accessory. A background scan was performed at the beginning of each round of analysis and every 120 minutes thereafter as per instrument instructions. Prior to the background scan, the crystal surface was cleaned with isopropanol and the pressure plate of the ATR accessory was removed from contact with the crystal surface. Scans were acquired in the wavelength range of 4000-400 cm^{-1} and reported graphically as percentage transmittance with a sensitivity rating of 50-55.

Solid samples (dried sludges and biosolids) were introduced using a metal spatula, placing a small quantity of homogenised sample on the crystal surface and applying the pressure plate of the ATR accessory. For liquid samples, a glass dropper pre-cleaned with isopropanol was used to apply a droplet of leachate onto the crystal surface. Due to the significant overlap of the indicative peaks of water on the FTIR spectra, liquid samples were applied to the crystal surface and left to dry completely before scans were performed with the spectra being collected from the residue. A liquid sample of 100 mg·L^{-1} KHP was used to confirm this approach was suitable for aqueous samples, with the sample spectra being compared to a reference spectrum for KHP (Appendix 9). A small quantity of isopropanol was used to clean the crystal surface between scans. Blank scans of the crystal surface were carried out following cleaning with isopropanol to confirm absence of contamination. Duplicate scans were performed for all samples to check for agreement, and possible contaminant carry-over. This helped to confirm that the dried residues of the liquid leachates provided a good repeatable representation of the indicative non-water peaks present. Peak identification was used to label dominant peaks with position and intensity recorded.

9.3 Results

9.3.1 UV-Vis

9.3.1.1 Wavelength scans

Figure 9-1 to Figure 9-8 show graphical presentations of the wavelength scans for leachates from DDOC and PDDOC experiments as day 0 (DDOC) and after 28 day aerobic biodegradation (D28) and 10 day anaerobic (D38) biodegradation. All scans indicate a similar profile for day 0 leachates, with a large number of small peaks in the UV range from 190-260 nm, followed by a sharp rise and peak in the 270 to 290 nm range, and a tailing off towards 400 nm. There is little indication of absorbance in the visible wavelength range (> 400 nm) for most samples. This provides some indication that the persistent fraction of PDDOC samples is not dominated by humic substances which would be observed in this higher wavelength range (Dizer *et al.* 2002).

All sites where day 28 and day 38 scans were completed show a sharp decreased in the 270-290 nm peak compared to day 0. The day 28 and day 38 wavelength scans do not show a vast difference indicating that the AD experiments had little impact on the leachates, which was also reflected in the PDDOC results.

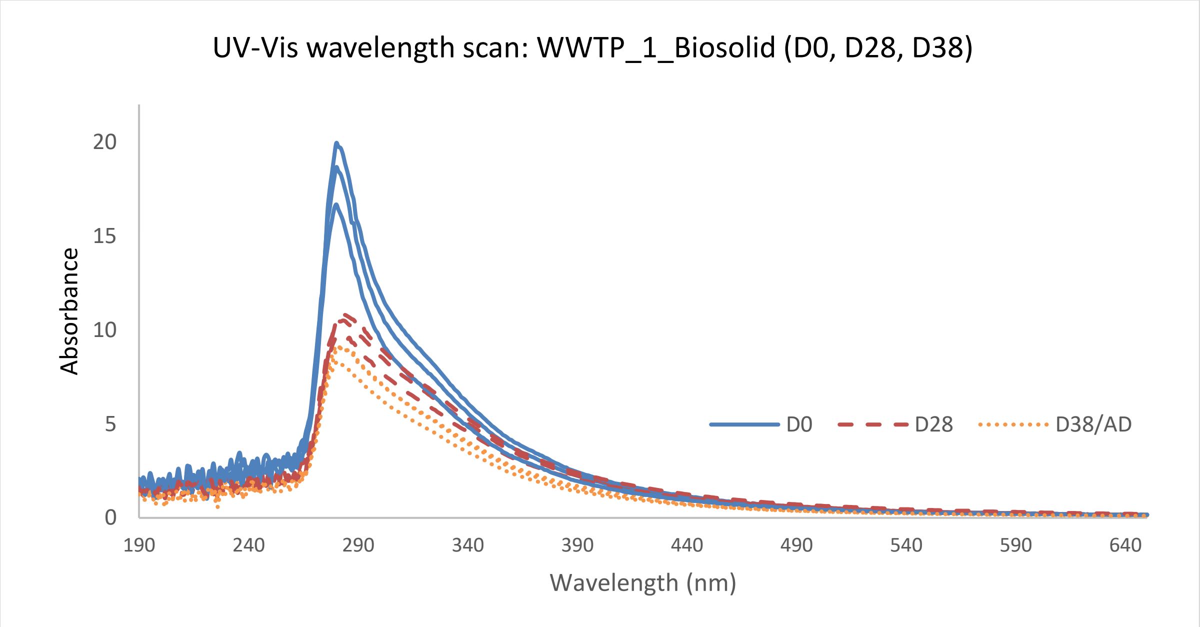


Figure 9-1 UV-Vis wavelength scan: WWTP_1, biosolid leachates for day 0 (D0), day 28 (D28) and day 38 (D38/AD).

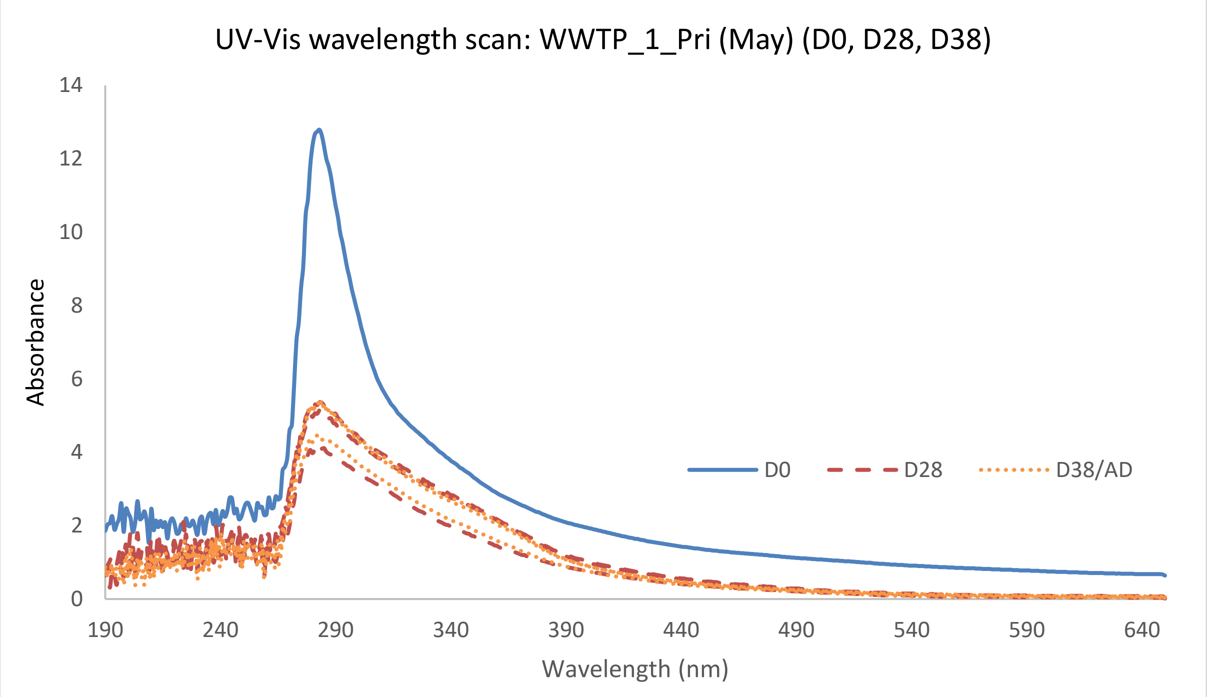


Figure 9-2 UV-Vis wavelength scan: WWTP_1, primary treatment leachates for day 0 (D0), day 28 (D28) and day 38 (D38/AD).

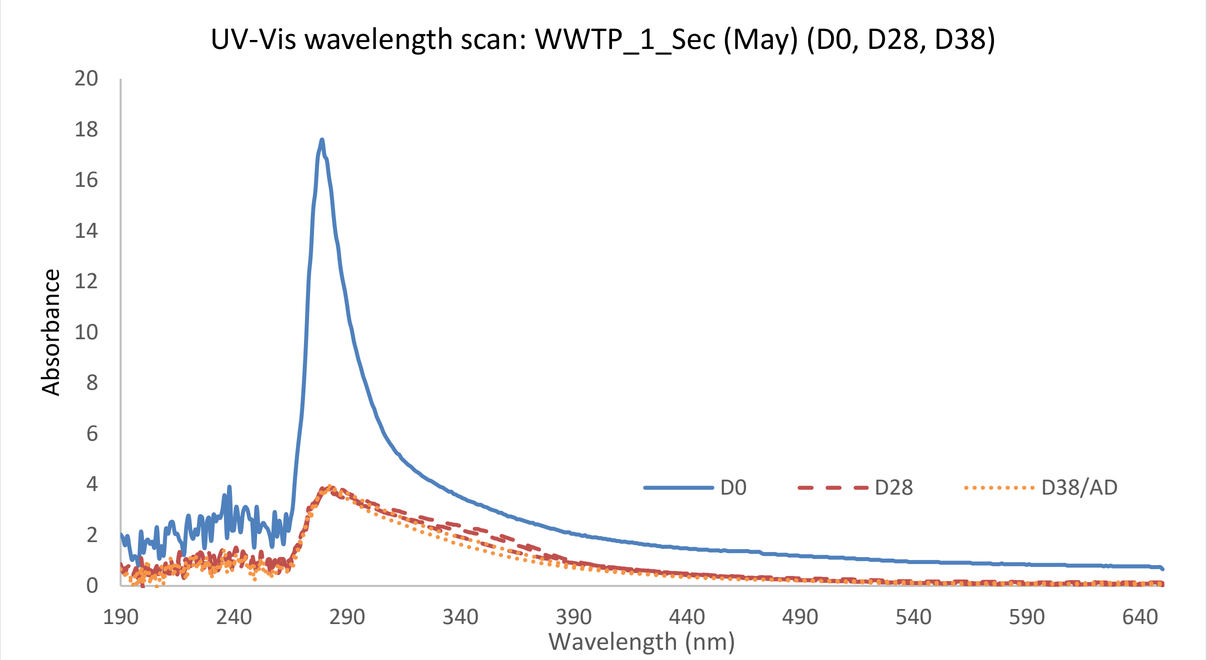


Figure 9-3 UV-Vis wavelength scan: WWTP_1, secondary treatment leachates for day 0 (D0), day 28 (D28) and day 38 (D38/AD).

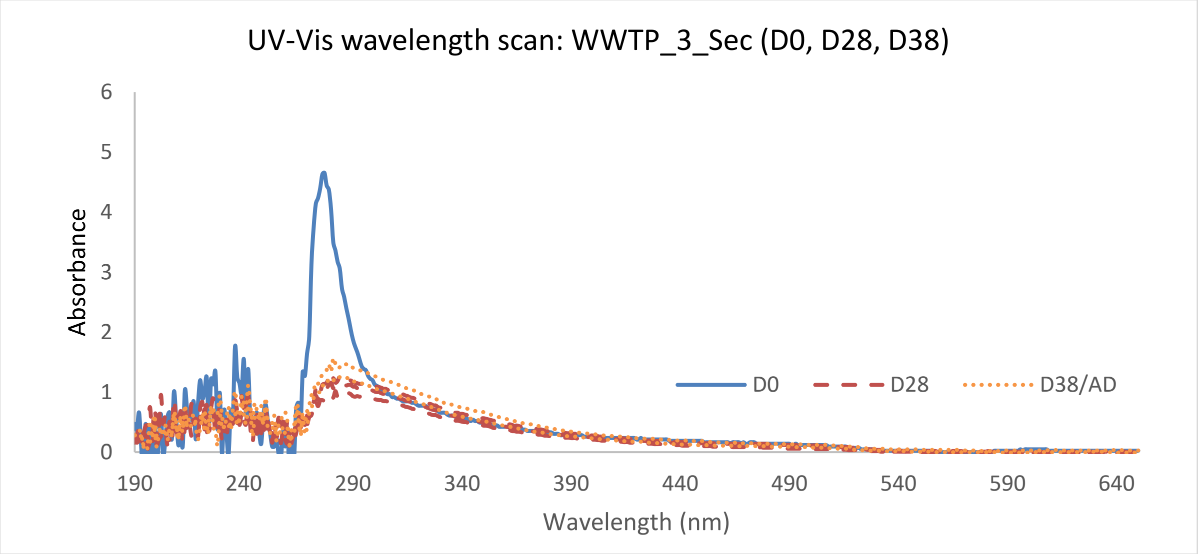


Figure 9-4 UV-Vis wavelength scan: WWTP_3, secondary treatment leachates for day 0 (D0), day 28 (D28) and day 28 (D38/AD).

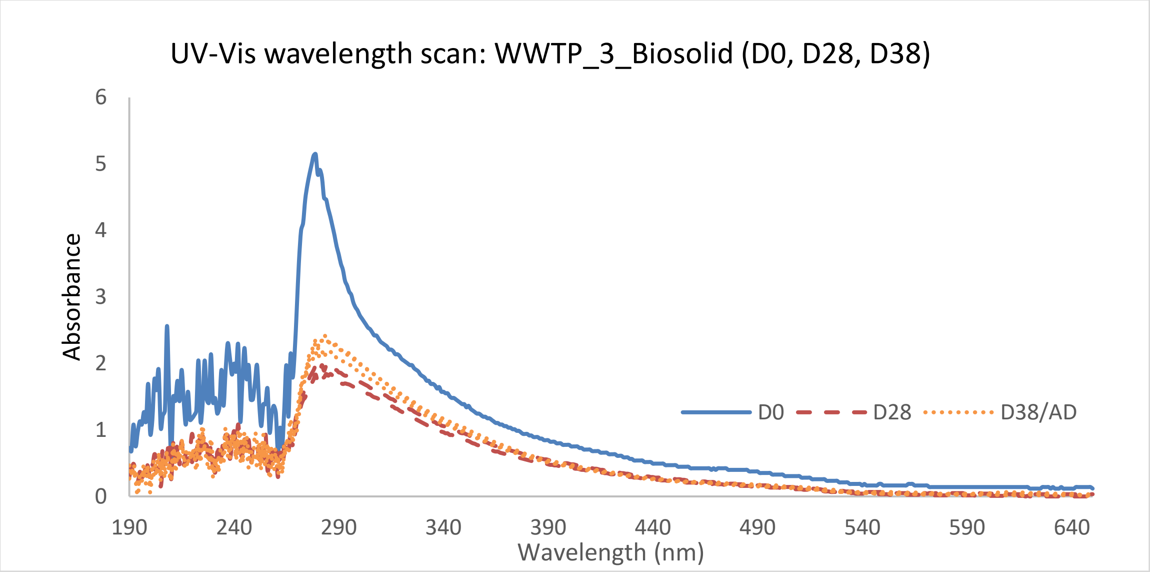


Figure 9-5 UV-Vis wavelength scan: WWTP_3 biosolid leachates for day 0 (D0), day 28 (D28) and day 38(D38/AD).

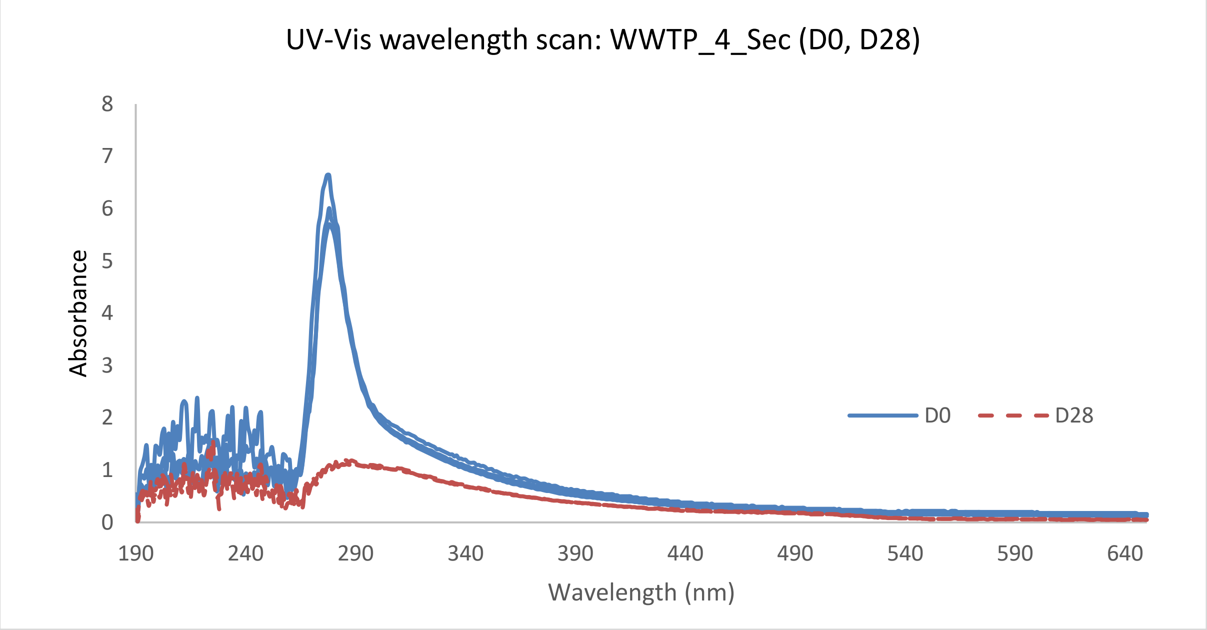


Figure 9-6 UV-Vis wavelength scan: WWTP_4, secondary treatment leachates for day 0 (D0) and day 28 (D28).

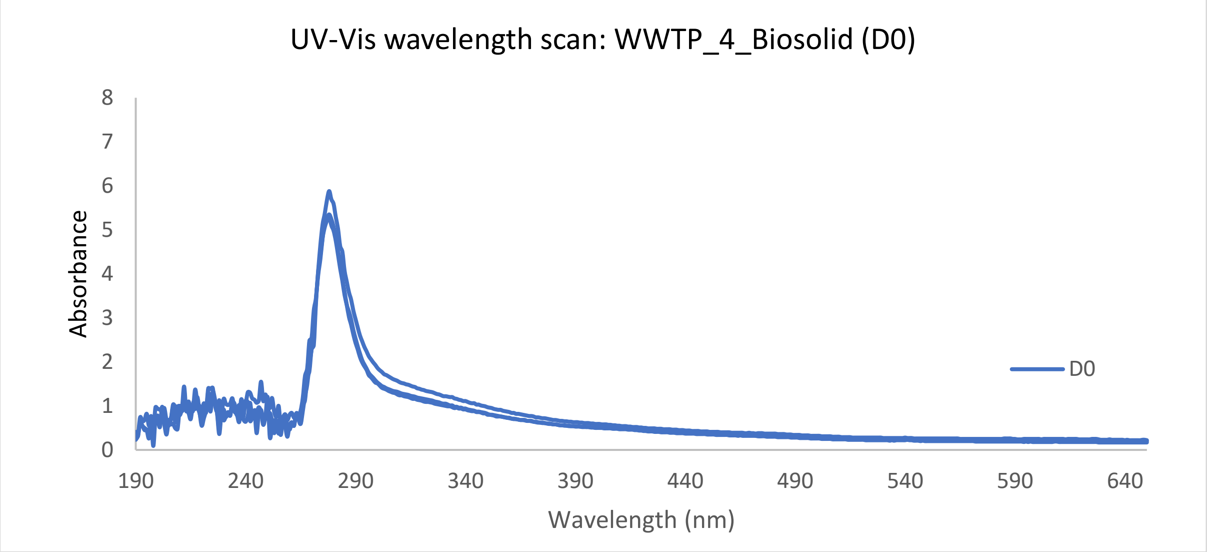


Figure 9-7 UV-Vis wavelength scan: WWTP_4, biosolid leachates for day 0 (D0).

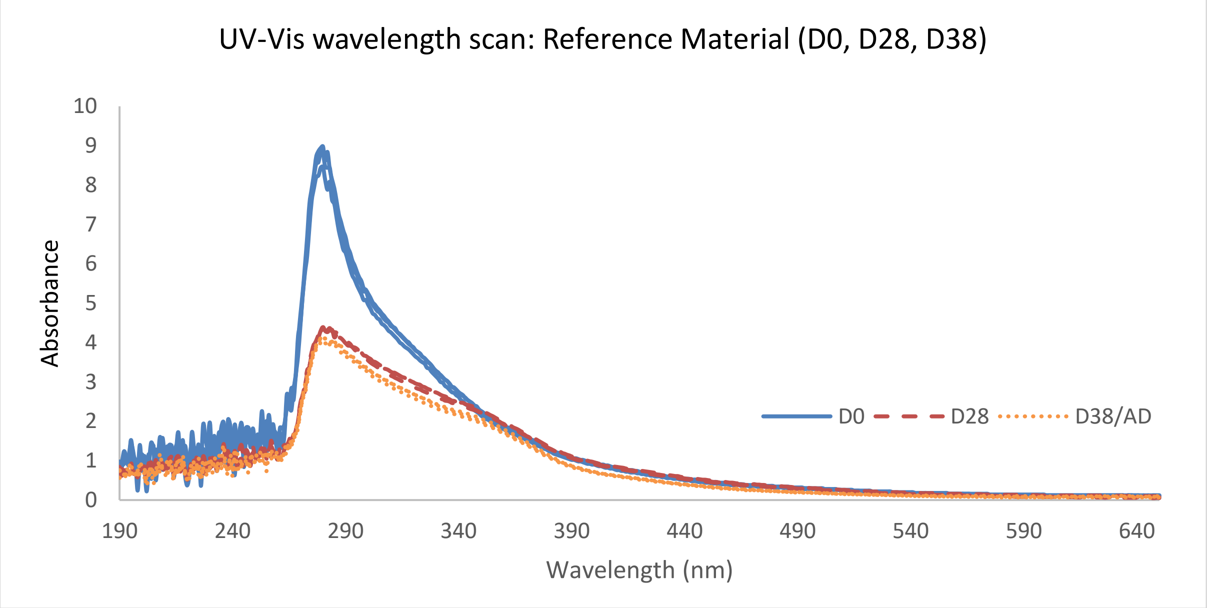


Figure 9-8 UV-Vis wavelength scan: Reference material leachates for day 0 (D0), day 28 (D28) and day 38 (D38/AD).

Along with the decrease in the 270-290 nm range, there is a distinct reduction in the lower wavelength absorbances, in contrast to higher wavelength absorbances for day 28 and day 38 following the profile of the day 0 samples quite closely. This is shown clearly in Figure 9-8. This provides some indication of the types of compounds that are remaining in the persistent fraction of the leachates. Longer wavelength absorbances are typically associated with higher molecular weight and more complex organic compounds with higher levels of conjugation.

It should be noted that a significant absorbance remains for all samples around 280 nm. This wavelength is representative of a wide range of compounds, which could include both readily biodegradable and poorly degradable compounds including PPCPs. It is therefore expected that although a reduction in this characteristic wavelength is observed as biodegradable compounds are removed, the peak would not be expected to disappear completely. For example, carbamazepine, known to be present in wastewater effluents (Al Qarni *et al.* 2016) and triclosan, known to be present in wastewater solids (Stansinakis 2012), have a peak UV absorbance at 285.5 nm 280 nm respectively.

9.3.1.2 Comparison of UV absorbance ratio E2:E3

Absorbance ratios comparing the ratio of two wavelengths have been used by others to examine rates of degradation and to understand the quality of the DOC in a measured sample (e.g. Saadi *et al.* 2006; Antilen *et al.* 2014; Li and Hur 2017) The ratio E2:E3, which is the absorbance ratio of absorbance at 250 nm and 365 nm, has been used to understand the relative aromaticity or molecular weight of the DOC fraction to provide an indication of the relative size of molecules in dissolved organic matter studies (De Haan and De Boer 1987; Helms *et al.* 2008; Peacock *et al.* 2014). E2:E3 decreases with increased molecular weight (MW), as larger molecules absorb light at higher wavelengths. The E2:E3 ratio was measured for all samples using mean absorbance with results presented in Table 9-1 sorted by the lowest to highest E2:E3 ratio on day 0. Samples from WWTP_1, the non-nitrifying large treatment plant, were found to have some of the lowest ratios, along with the reference material biosolid at day 0. This suggests that the DDOC from this site contains higher MW molecules compared to the other sites. By day 28, all sites indicated a reduction in the E2:E3 ratio, apart from WWTP_4 secondary leachate, signalling an increase in the proportion of high MW compounds for most sites in PDDOC. By day 38, the ratio increased further for some sites. The ratio for WWTP_3 for biosolids was lower than for secondary sludge, indicating a higher concentration of high MW compounds in the biosolid leachate. The day 28 and day 38 ratios of the nitrifying and partially nitrifying plants are higher than the non-nitrifying plant suggesting that the non-nitrifying plant has a higher concentration of high MW compounds.

Table 9-1 E2:E3 ratios (250:365 nm) for WWTP_1, 3, 4 and Reference material biosolid on day 0 (D0), day 28 (D28) and day 38 (D38)

Sample ID	**E2:E3**		
	D0	**D28**	**D38**
WWTP_1_Biosolid	0.66	0.54	0.74
Ref_Biosolid	0.74	0.56	0.64
WWTP_1_Pri	0.81	0.79	0.72
WWTP_1_Sec	1.08	0.52	0.64
WWTP_4_Sec	1.31	1.42	n.d.
WWTP_4_Biosolid	1.40	n.d.	n.d.
WWTP_3_Biosolid	1.55	1.12	0.93
WWTP_3_Sec	2.00	1.58	1.84

n.d. = no data

9.3.1.3 Comparison of Specific UV absorbance (SUVA)

Weishaar *et al.* (2003) are cited widely in the literature as demonstrating the use of the specific UV absorbance to estimate the aromaticity of dissolved organic matter, finding that UV absorbances at 254 nm normalised to the dissolved organic carbon (DOC) concentration showed a strong correlation with aromaticity of the solution. This value ($SUVA_{254}$) was calculated as described earlier using mean absorbances at 254 nm and DOC concentrations for day 0, day 28 and day 38. A graphical comparison of $SUVA_{254}$ for leachates from primary and secondary sludges and biosolids is presented in Figure 9-9 and Figure 9-10. The comparison of primary and secondary sludge leachates indicates that the day 0 $SUVA_{254}$ was highest for the primary sludge, with all sites increasing by day 28, and a slight decrease on day 38. The secondary sludge from the non-nitrifying plant was lower than for the nitrifying and part-nitrifying plant and the primary sludge. The comparison of biosolids (Figure 9-10) shows a similar pattern to Figure 9-9 with an increase in $SUVA_{254}$ between day 0 and day 28 for all samples. There is no clear pattern of increase or decrease for day 38. The comparison between biosolids and the primary and secondary sludges reveals more variability amongst the primary and secondary sludges compared to the biosolids.

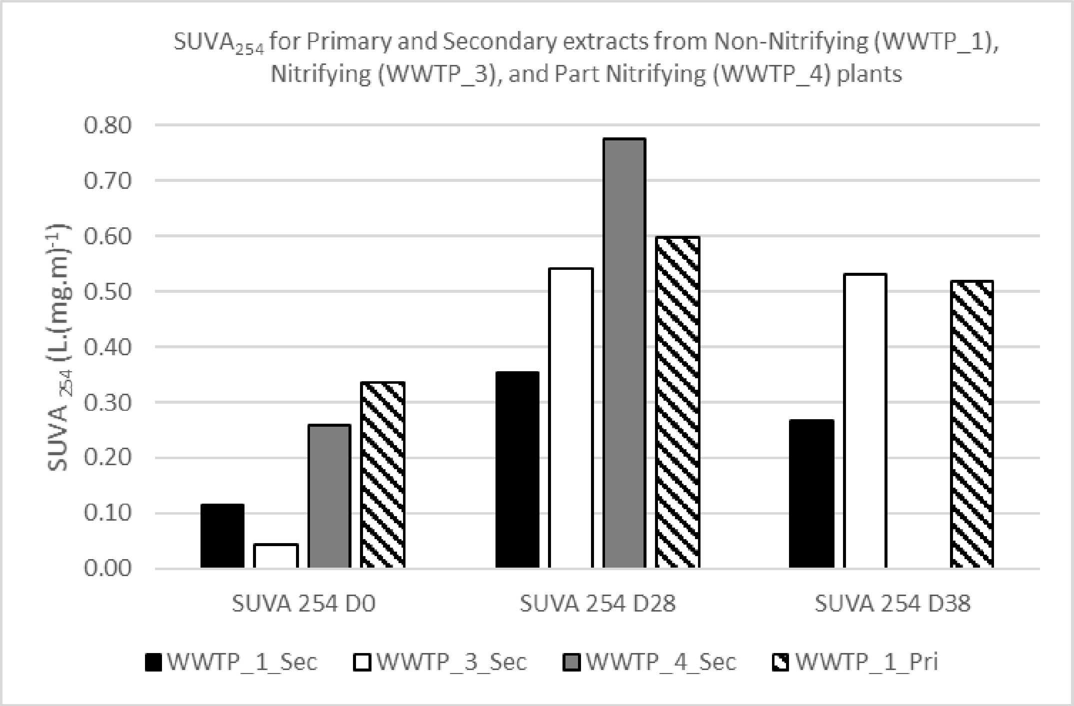


Figure 9-9 Specific UV absorbance at 254 nm for WWTP_1, 3 and 4 at day 0 (D0), day 28 (D28) and day 38 (D38).

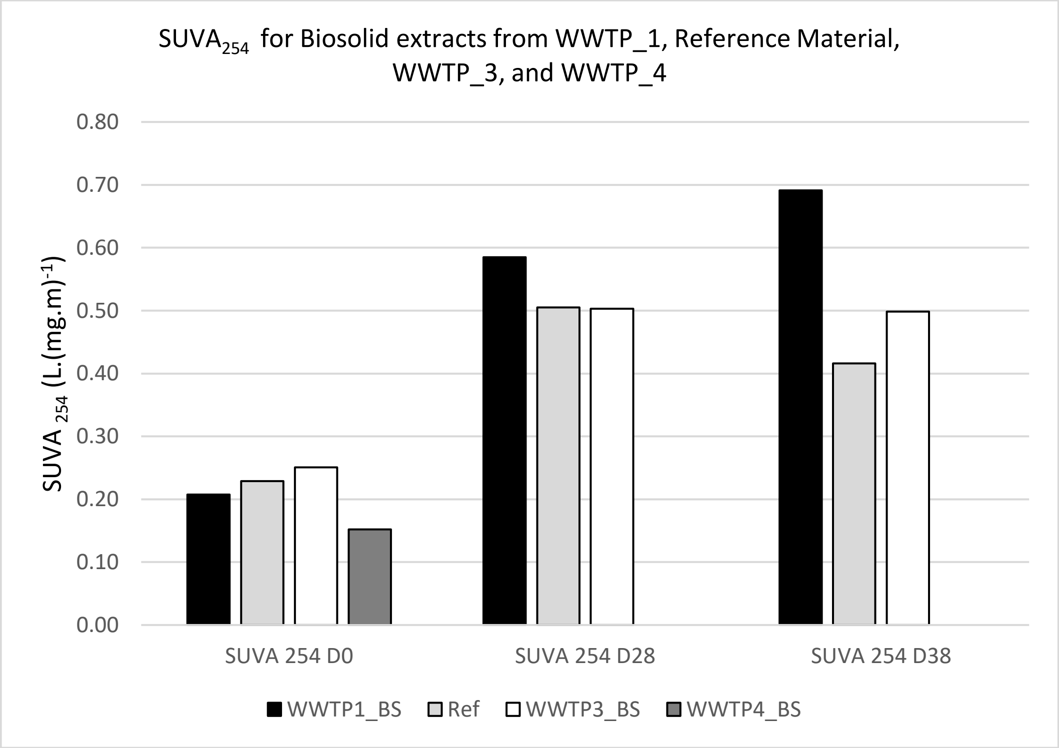


Figure 9-10 Specific UV absorbance at 254 nm for WWTP_1, 3 and 4 and Reference Material at day 0 (D0), day 28 (D28) and day 38 (D38)

9.3.2 FTIR

A selection of FTIR spectra is presented below to highlight some of the key features identified for biosolids and for aqueous leachates from DDOC and PDDOC experiments.

9.3.2.1 FTIR spectra: Solids

Figure 9-11 to Figure 9-19 present the FTIR spectra for dried sludges and biosolids used in the study. The spectra are arranged by WWTP site. No remaining material was available for WWTP_2 primary dry sludge due to the small initial sample size being entirely used in the DDOC experiments. Only one replicate per sludge type is presented below, with full results presented in Appendix 10.

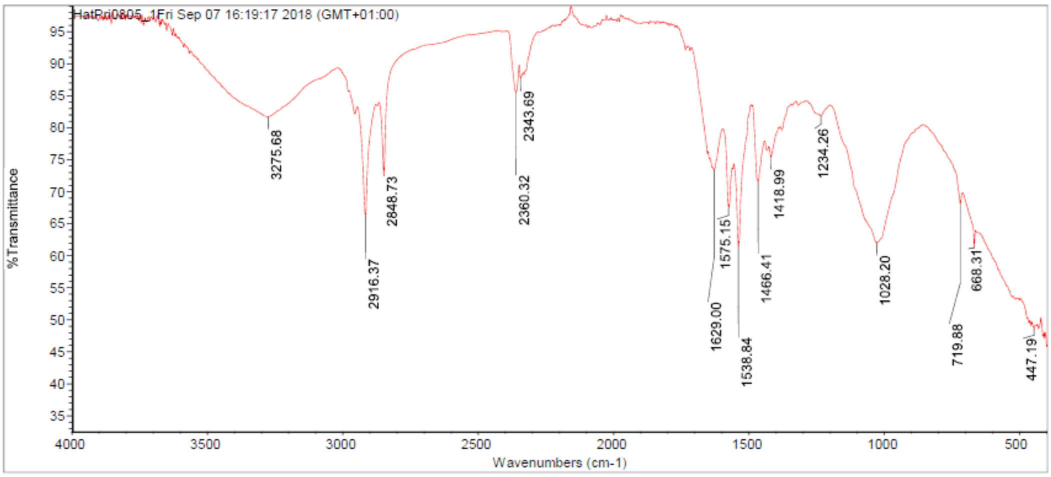


Figure 9-11 FTIR spectra for WWTP_1 primary (May), dried sludge

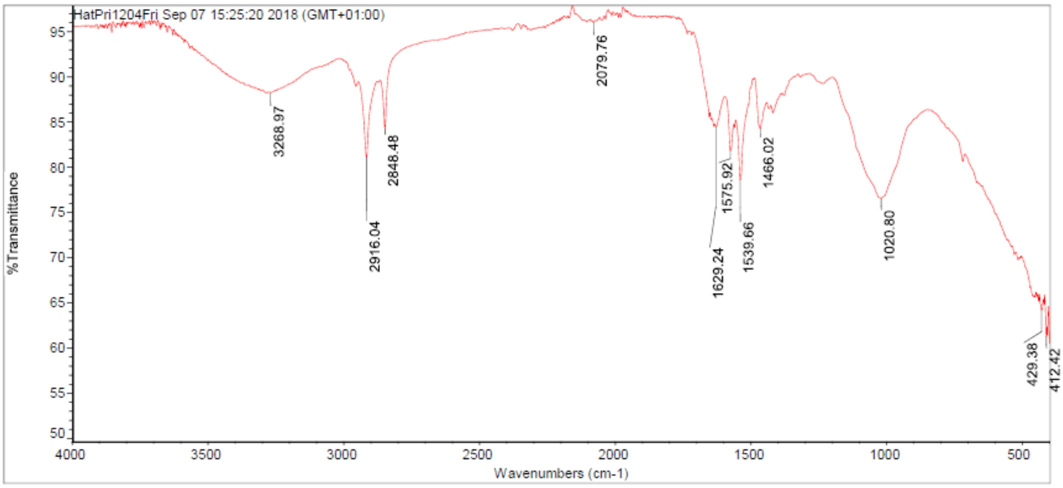


Figure 9-12 FTIR spectra for WWTP_1 primary (Apr), dried sludge

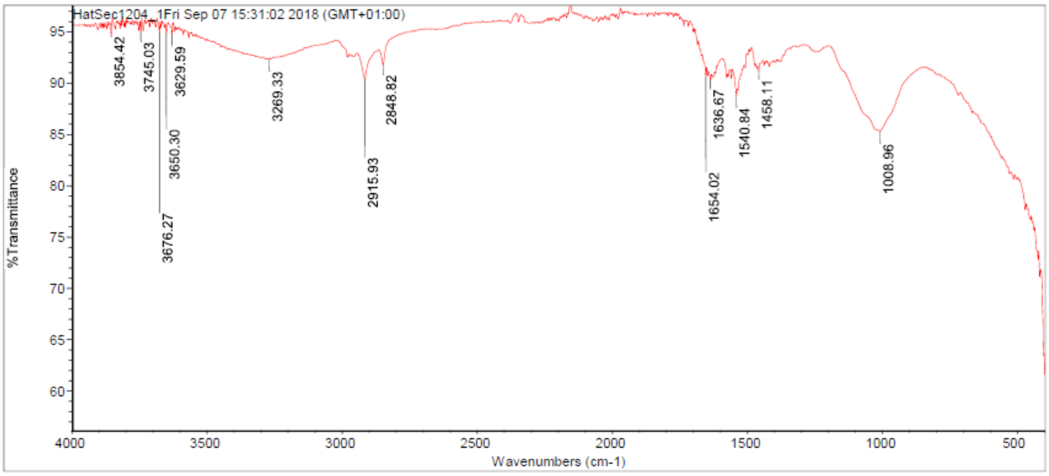


Figure 9-13 FTIR spectra for WWTP_1 secondary (Apr), dried sludge

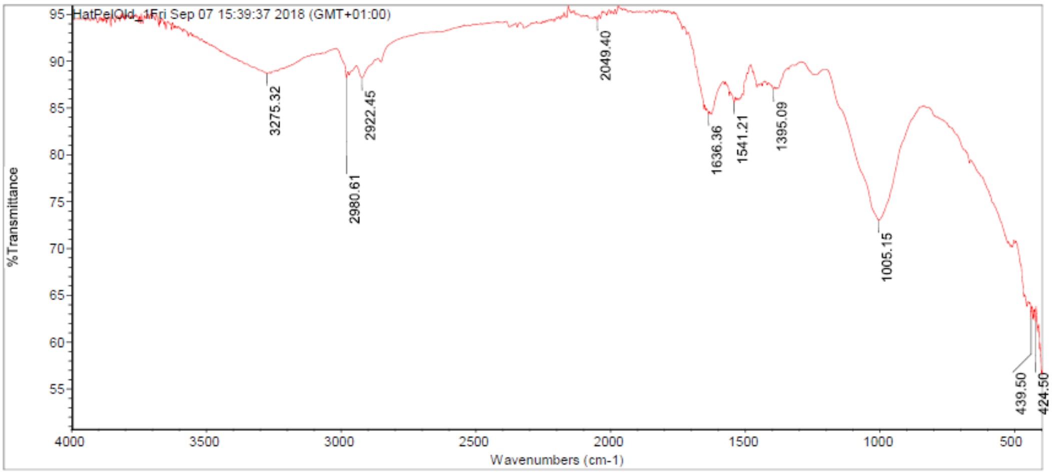


Figure 9-14 FTIR spectra for WWTP_1 pellets, dried biosolid

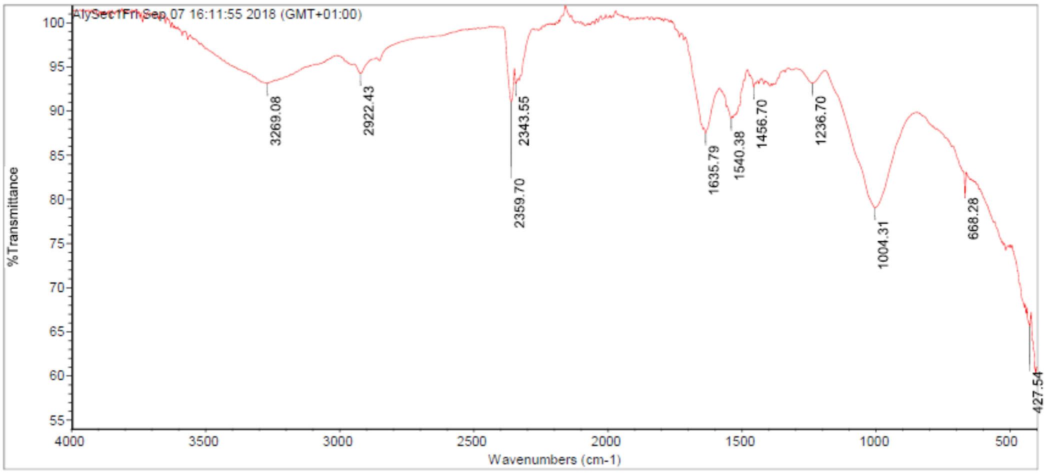


Figure 9-15 FTIR spectra for WWTP_2 secondary, dried sludge

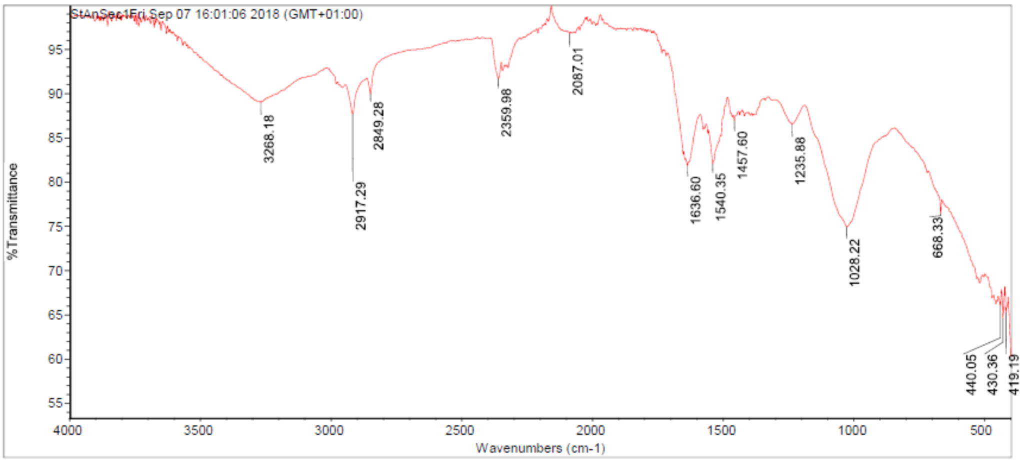


Figure 9-16 FTIR spectra for WWTP_3 secondary, dried sludge

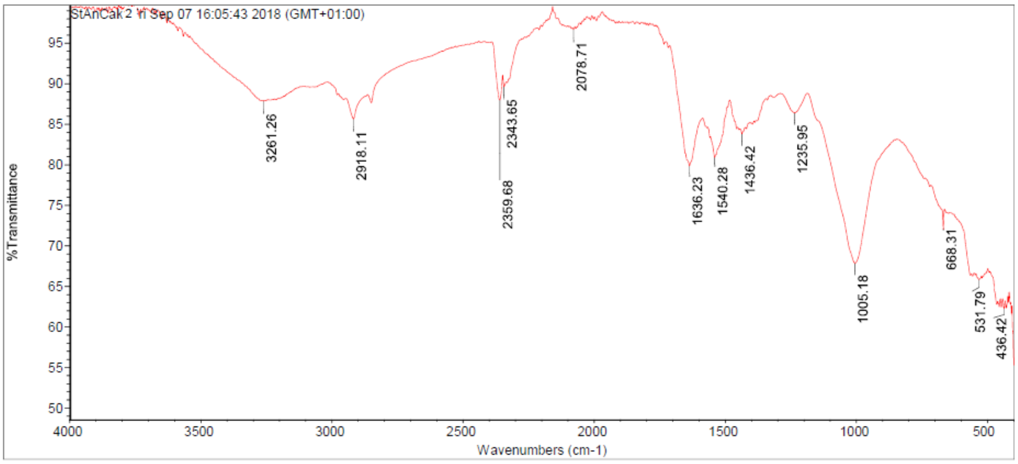


Figure 9-17 FTIR spectra for WWTP_3 sludge cake, dried biosolid

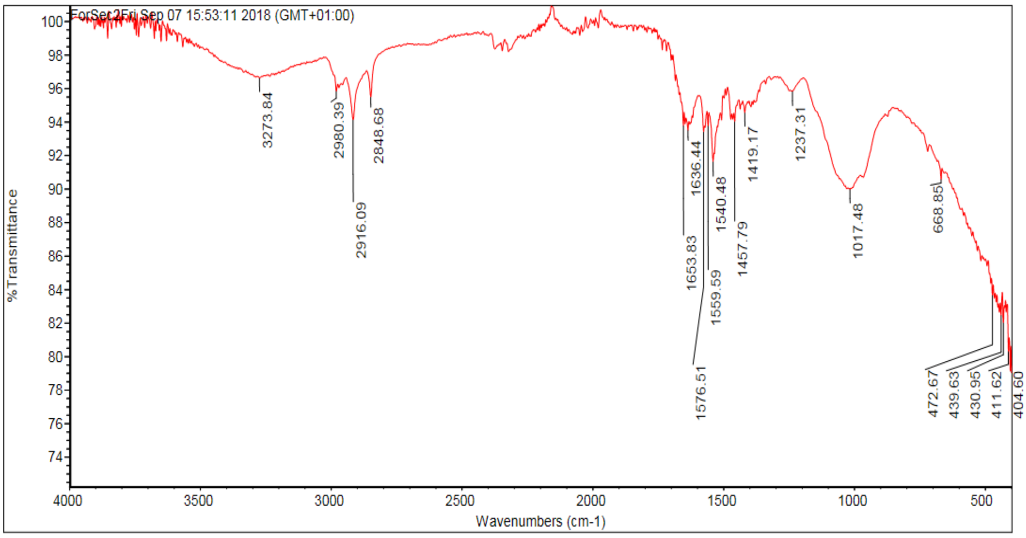


Figure 9-18 FTIR spectra for WWTP_4 secondary, dried sludge

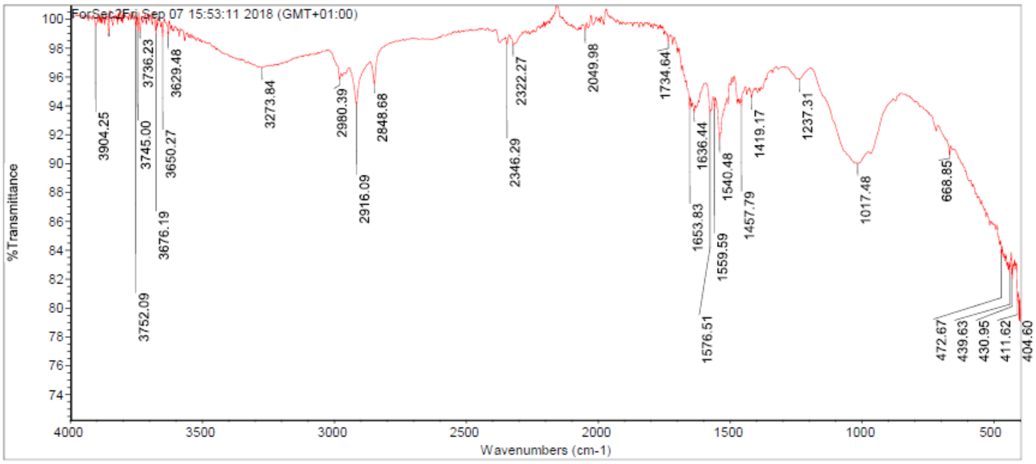


Figure 9-19 FTIR spectra for WWTP_4 sludge cake, dried biosolid

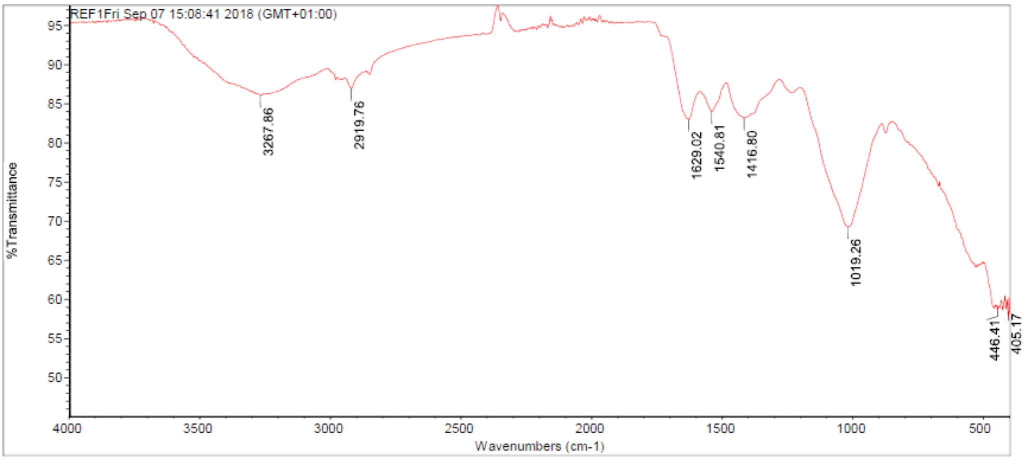


Figure 9-20 FTIR spectra for Reference material, dried biosolid

9.3.2.2 FTIR spectra: Aqueous leachates.

The analysis of aqueous leachates using the drying method was found to be suitable for leachates with sufficient remaining sample volume, and samples with the highest starting concentration of DOC. Figure 9-21 to Figure 9-26 present the spectra showing progression from day 0 (D0), to day 28 (D28) and finally day 38 (D38) for comparison.

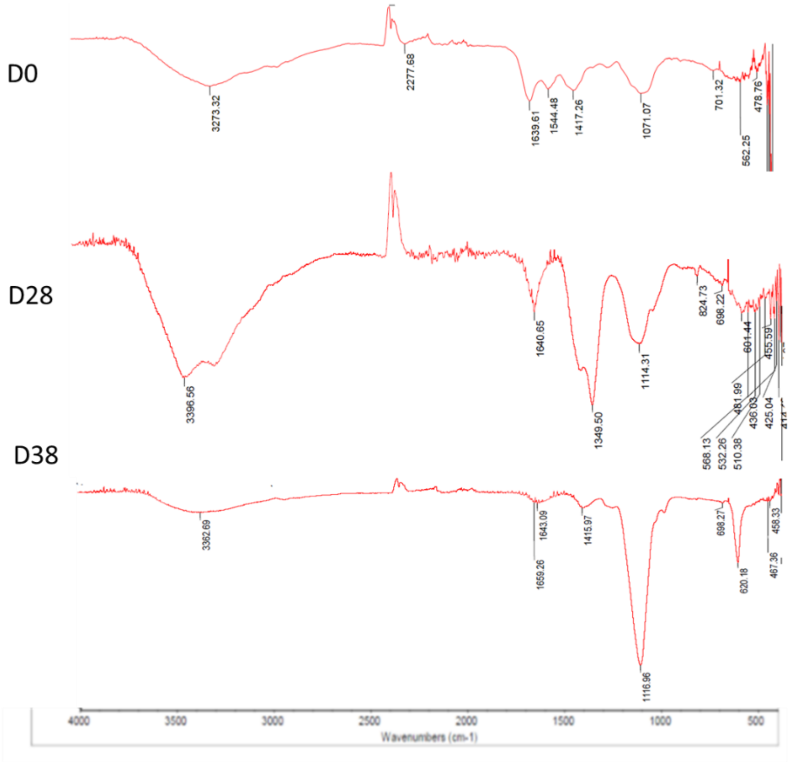


Figure 9-21 FTIR Spectra for WWTP_1 primary sludge leachates day 0 (D0), day 28 (D28) and day 38 (D38)

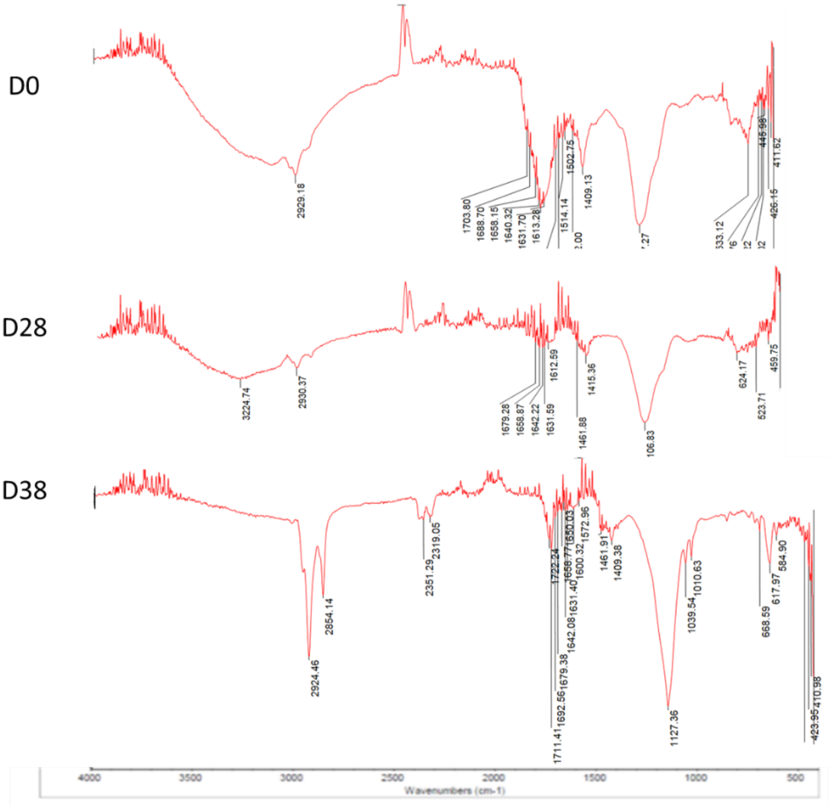


Figure 9-22 FTIR Spectra for WWTP_1 secondary sludge leachates for day 0 (D0), day 28 (D28) and day 38 (D38)

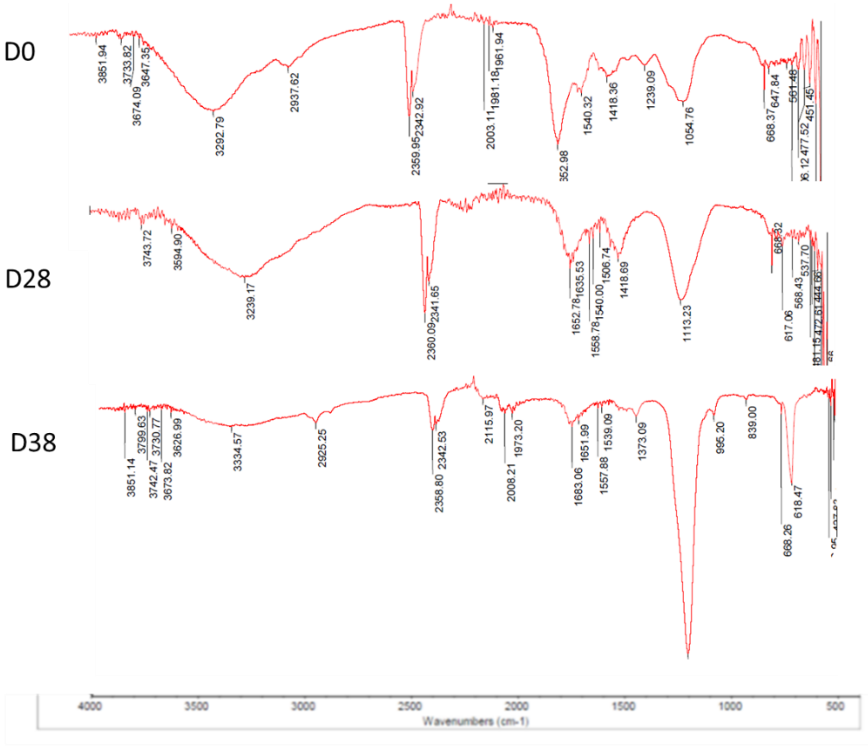


Figure 9-23 FTIR Spectra for WWTP_1 biosolid leachates for day 0 (D0), day 28 (D28) and day 38 (D38)

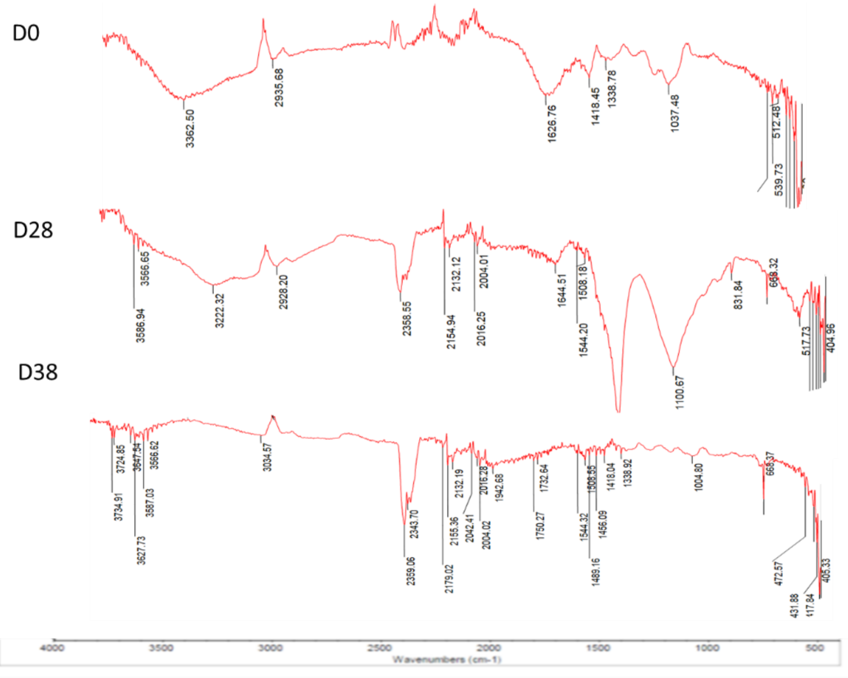


Figure 9-24 FTIR Spectra for WWTP_3 secondary sludge leachates for day 0 (D0), day 28 (D28) and day 38 (D38)

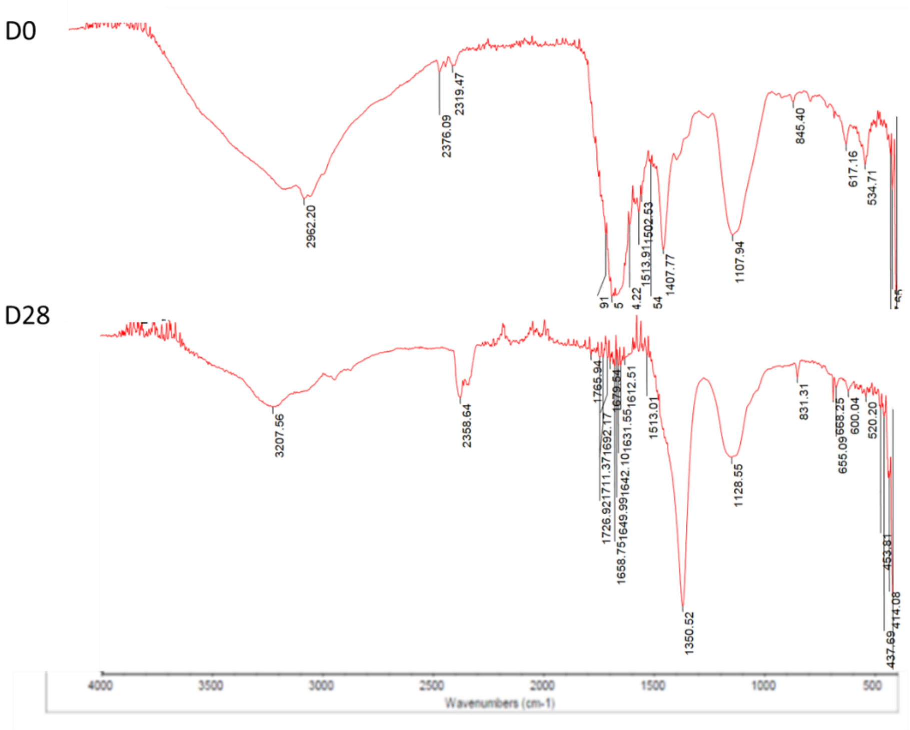


Figure 9-25 FTIR spectra for WWTP_4 secondary sludge leachates for day 0 (D0), and day 28 (D28)

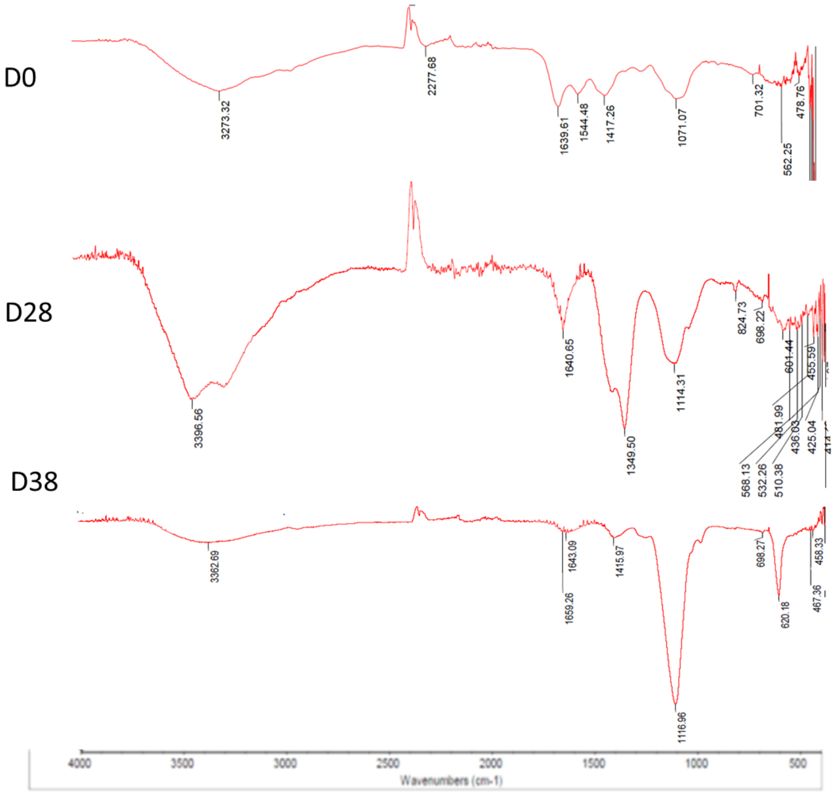


Figure 9-26 FTIR Spectra for Reference material leachates for day 0 (D0), day 28 (D28) and day 38 (D38)

9.4 Discussion

The previous experiments provide some indication of the types of compounds that would be predicted to be present in sludges and biosolids at each stage of the biodegradation study. The day 0 samples representing the desorbed DOC before biodegradation processes are expected to be dominated by typically mobile compounds. This includes the less hydrophobic compounds (e.g. low log K_{ow}) or those with a high desorption coefficient (D_{ow}) (Barbosa *et al.* 2016) and intermediately soluble compounds (Duarte-Davidson and Jones 1996) such as some organic acids, proteins, and acidic pharmaceuticals (e.g. clofibric acid, ibuprofen, gemfibrozil, naproxen, diclofenac) (Kinney *et al.* 2006; Gibson *et al.* 2010). Other compounds that may be likely to dominate scans include surfactants, which are typically present at high concentrations in biosolids due to ubiquitous use in society (Stansinakis 2012). Surfactant groups can include anionic (linear alkyl benzene sulphonates, LAS) non-ionic (NPE) and cationic (quaternary ammonium-based compounds). Some personal care products found to be present at high concentrations in biosolids such as triclosan, triclocarban, and galaxolide may also be present (Heidler and Halden 2009).

Some compound types expected to be present at day 0 will biodegrade or bio-transform by day 28, whereas more persistent compounds may become more prevalent as the easily biodegradable compounds are removed. Surfactants such as LAS are likely to biodegrade when present at low concentrations, and pharmaceuticals such as NPE show rapid biotransformation to degradation products (e.g. nonylphenol, nonylphenol monoethoxylate, and nonylphenol diethoxylate) (Stansinakis 2012). Halogenated compounds are more likely to resist degradation. For example, chlorinated aromatic compound degradation is inhibited by the chloro-group and the rate of degradation decreases with increasing chlorination (Wang and Jones 1994). Halogenated compounds such as PBDEs and PFOS have been widely detected at high concentrations in sludges in the literature, with bromo- and fluoro-groups contributing to non-biodegradability. Indications of these compounds in sludge and biosolid leachates would not be surprising, however further characterisation would be needed to confirm their presence (Eljarrat *et al.* 2016).

9.4.1 UV-Vis

9.4.1.1 Wavelength scans

The results of the wavelength scan provide an overview of dominant wavelengths in the initial leachate (D0), after 28 days aerobic biodegradation (D28) and after an additional 10 days of anaerobic biodegradation (D38). Although the previous experiments allowed for comparison of DOC degradation, there was little indication of the nature of the DOC in the starting material and the remaining (persistent) fraction. The wavelength scans for all sites indicate that day 0 samples are dominated by the 280 nm peak, with significant absorbance noted between the 260-300 nm range. There are many compounds that could be represented at these wavelengths; however, 280 nm is often dominated by protein-like structures including DNA and easily biodegradable substances such as carbohydrates and fatty acids (Saadi *et al.* 2006). In wastewater treatment processes, absorbance at 280 nm has been found to provide a reasonable prediction of 5 day biological oxygen demand (BOD_5) indicating a good relationship with readily biodegradable substances (Mesquita *et al.* 2017).

The profiles for the degradation process indicate that day 28 and day 38 leachates have significantly reduced absorbances around 280 nm, which would indicate reduction in readily biodegradable compounds as expected. The wavelength scans indicate that there is not a significant decrease in relative absorbance at the higher wavelengths, for example those above 300 nm. Absorbances at these higher wavelengths can relate to compounds with greater conjugation representing more complex longer chain molecules (Kumar 2006). This suggests the presence of more persistent substances that have not been degraded by either the aerobic or anaerobic conditions. Absorbances at these wavelengths may also be due to inorganic materials, that remain unchanged by biodegradation processes.

The comparison of wavelength scans for day 28 and day 38 leachates reveals similar profiles, which aligns with the findings of the PDDOC experiments presented in Chapter 8 that indicated that the additional 10 d anaerobic biodegradation experiments had little impact on additional reduction in DOC, with slight increases noted in samples tested (WWTP_1, WWTP_3 and Reference material). The shape of profiles is similar above 300 nm; however, some differences are noted in the lower wavelengths, indicating that different break-down products may be present.

For WWTP_3 biosolids, PDDOC results had indicated a large difference between day 28 and day 38 results with PDDOC increasing following the anaerobic experiment. This unusual result can be confirmed as possible contamination, with the UV wavelength profiles showing higher absorbances in the 270-300 nm range for the D38 samples compared to the D28 samples as indicated in Figure 9-27. This result helps to rule out instrumental error in the analysis of DOC in the leachates.

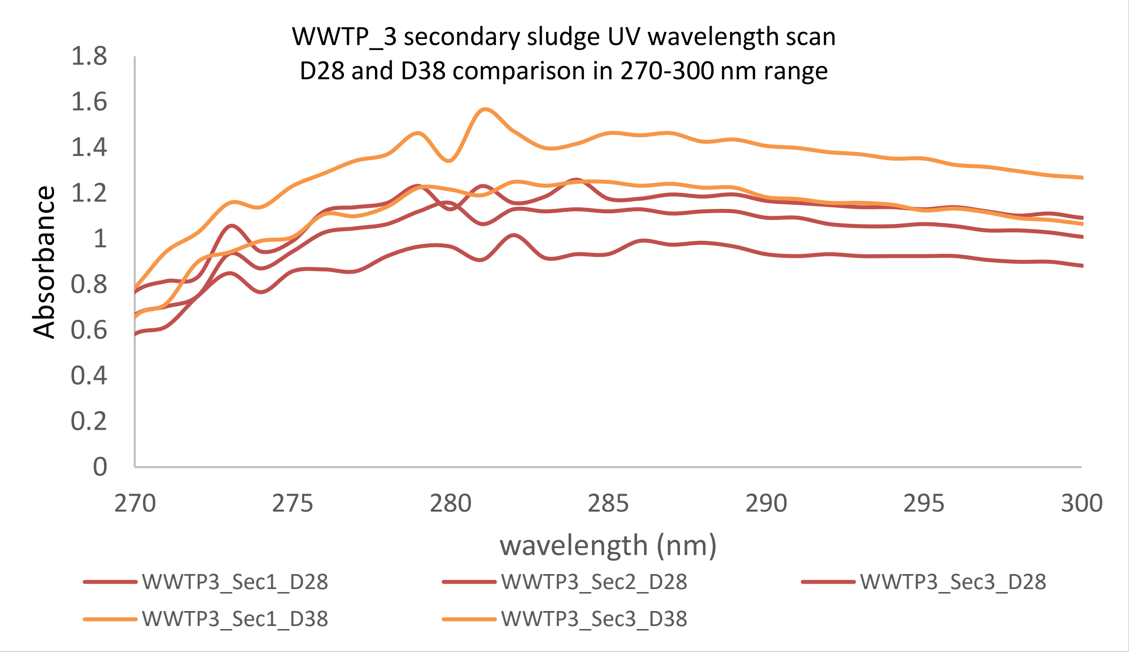


Figure 9-27 WWTP_3 secondary sludge wavelength scans for day 28 (D28) and day 38 (D38) leachates between 270-300 nm

9.4.1.2 Absorbance ratios

E2:E3 has been found to negatively correlate with MW and aromaticity in the literature (Li and Hur 2017). The comparison of E2:E3 ratios in this study indicates that the reference material and the non-nitrifying plant biosolids had the lowest ratios for all sample types examined. This suggests that the additional treatment of these samples (thermal treatment and AD) has resulted in a biosolid leachate with a higher proportion of high MW compounds. There are lower E2:E3 ratios for the non-nitrifying plant secondary sludges (WWTP_1) compared to the part nitrifying (WWTP_4) and nitrifying secondary leachates (WWTP_3). This suggests that the additional biological treatment may have been effective in reducing the proportion of high MW compounds in the leachates. This may provide some indication of the nature of the persistent material that remains in the leachates from nitrifying and partially-nitrifying plants, with the persistent material dominated by lower MW compounds as compared to the non-nitrifying plant. The results for the reference material are similar to that of non-nitrifying plant. This material has undergone thermal treatment, which may have been effective in reducing some of the low MW compounds, with the larger MW compounds being more difficult to degrade. There may also be differences in the source catchments for WWTP_1 and the reference material that result in a higher proportion of high MW compounds being present, with WWTP_1 known to receive effluent from a large hospital and industrial inputs at this site.

$SUVA_{254}$ has been found to be positively correlated with aromaticity, DOC hydrophobicity and MW (Weishaar *et al.* 2003). The comparison of primary and secondary sludge leachates found that day 0 $SUVA_{254}$ was highest for the primary sludge, indicating a higher proportion of aromatic, hydrophobic and high MW compounds in primary sludge leachate. Following the 28 day biodegradation test, the $SUVA_{254}$ increased for both the primary and secondary sludge leachates, with the partially-nitrifying plant secondary sludge having the highest $SUVA_{254}$. This may be due to the partially-nitrifying plant having no primary treatment, therefore compounds that could have been removed in primary treatment are instead carried through to the activated sludge tank. The non-nitrifying plant, which had primary settlement, had the lowest $SUVA_{254}$ in secondary sludge after day 28, although the primary sludge had the second highest, indicating that primary treatment at this plant may have been responsible for removing some of the aromatic, hydrophobic and high MW compounds at this plant. Trends for day 38 were less clear, as both increases and decreases were observed for some samples and no clear pattern was observed.

The comparison of $SUVA_{254}$ in the biosolid leachates also showed an increase from day 0 to day 28, indicating an increase in aromatic, hydrophobic and high MW compounds from DDOC to PDDOC, which agrees with the results of the E2:E3 comparison. There was less difference observed between the treatment plants, with the non-nitrifying plant having a slightly higher $SUVA_{254}$ at D28 compared to the reference material and the nitrifying plant biosolid leachate. This adds evidence to the suggestion that conventional wastewater treatment and sludge treatment processes, although showing slight differences, result in a similar magnitude of persistent organic compounds remaining in leachates.

Calculation of $SUVA_{254}$ in this study found that the results were not comparable to those from literature, which are typically much higher, however, most of the results from literatures are based on environmental waters, soil leachates, or treated municipal wastewater. Few researchers have examined these absorbance ratios for biosolid leachates, which have a significantly higher DOC concentration compared to treated municipal wastewater or environmental waters, resulting in a lower $SUVA_{254}$ value. For example, Yates *et al.* (2019) measured $SUVA_{254}$ in treated effluent from four wastewater treatment facilities in the UK, with measured DOC concentrations of 1.6–11.8 $mg{\cdot}L^{-1}$ DOC. This level of DOC is much lower than the results of this study, which measured between 80-1574 $mg{\cdot}L^{-1}$ DOC in leachates. This corresponded to $SUVA_{254}$ in the study by Yates *et al.* (2019) of between 2.5 and 2.8, compared to $SUVA_{254}$ in this study of 0.04-0.78. Environmental waters have been found to have ratios that may be even higher than those observed for municipal wastewater effluents (Peacock *et al.* 2014). Organic matter sources from biosolid may contain higher levels of fatty acids, amino acids and proteins, and paraffinic groups, so established absorbance ratios for soils, landfill leachates or environmental waters are not readily comparable to those found in biosolid leachates. This study is therefore one of the first applying the use of optical indices to characterise biosolid leachates and provides a useful benchmark for future studies.

9.4.2 FTIR

Chemical structure plays an important role in resistance to biodegradation. More complex compounds are less biodegradable (monocyclic vs polycyclic) and the presence of functional groups such as halogens reduces biodegradability. Compounds with linear structures, absent or short side chains, and unsaturated aliphatic compounds are more easily degraded. Less degradable compounds are typically larger and more complex. This complexity could include highly branched side chains, presence of sulphates or halogens, and saturated or polycyclic compounds (Luo *et al.* 2014). The purpose of analysing the solids and the leachates using FTIR in this study was not to identify individual compounds but to gain a better understanding of the general class of compounds and possible characteristic peaks identified for solids and leachates. The aqueous leachates were also analysed to determine if evidence of a shift in dominance of different types of functional groups or compound structures could be detected between day 0 and day 28. Several resources were referred to in interpretation of FTIR spectra (Wolkers and Oldenhof 2005; de Oliveira Silva *et al.* 2012; Simonescu 2012; Reinhart and Bolyard 2015; Singh *et al.* 2017).

9.4.2.1 Solids

The FTIR spectra for all dried solid samples examined produced a characteristic profile that was similar for all samples as shown in Figure 9-11 to Figure 9-19. This included key stretches at 3600-3000 cm^{-1} indicating the stretch of -OH groups found in alcohols and acids, a stretch at 2920 cm^{-1} indicating asymmetrical stretching of C-H bonds from methyl and methylene groups, a stretch at around 1630-40 cm^{-1} possibly indicating C-O double bond of primary amides, and also around 1540 cm^{-1}, an indication of amide compounds (de Oliveira Silva *et al.* 2012). Additional stretches in the regions of 3000-2800 cm^{-1} could be indicative of hydrocarbons. These findings are similar to those of de Oliveira Silva *et al.* (2012), whose FTIR spectra of WWTP sludge also showed these characteristic bands, with some minimal variations. The de Oliveira Silva *et al.* (2012) study notes a shoulder in the 3040 cm^{-1} due to C-H stretching, and an absorbance band at 3440 cm^{-1} attributed to N-H stretch, which was not observed in this study. The de Oliveira Silva *et al.* (2012) study did not mention the stretches at around 1050-1010 cm^{-1}, however, they are observed in the reported spectra, and seen in this study. This could indicate the presence of inorganic sulphur compounds and minerals that have silicon oxygen bonds. Interestingly, the spectra for solids from all treatment plants, and all stages of treatment have the same characteristic appearance, with no notable differences observed. Several of the key stretches identified also correlate with the FTIR spectra for LAS, observed by Mottaleb (1999) and may give an indication of the type of dominant structures present. Given that there are few studies that have characterised the FTIR spectra of municipal wastewater treatment sludges and biosolids, this study provides supportive evidence of characteristic appearance, which includes mainly aliphatic chains with double bonds as well as carbonyl, hydroxyl and amide groups consistent with mixed organic matter including readily biodegradable compounds including fatty acids, carbohydrates and proteins. These groups dominate all the solids, therefore flood the signal compared to specific organic compounds that are present in comparatively low concentrations.

9.4.2.2 Aqueous leachates (day 0, day 28, day 38)

The nature of the liquid sample and concentration of leachates appeared to have been a limiting factor for identifying clear indicators of compound structures in some samples, particularly day 28 and day 38 leachates (e.g. Figure 9-24), which were typically more dilute than day 0 leachates. The FTIR spectra for WWTP_3 sludge cake leachates were similarly too dilute to provide a clear spectrum with discernible stretches, making clear comparisons between day 0, day 28 and day 38 impossible. For the other spectra, there are some notable trends that can be examined further.

9.4.2.2.1 WWTP_1_Primary

As shown in Figure 9-21, the characteristic stretch around 3270 cm^{-1} seen in the solid samples is also seen in the day 0 sample spectra. This peak shifts in the day 28 sample towards a higher wavenumber and deeper peak, still within the 3600-3000 cm^{-1} range characteristic of -OH groups. Stretching above 3000 cm^{-1} can indicate vinyl or aromatic hydrogen, and when paired with peaks at 1600-1450 cm^{-1}, and weak peaks at 2000-1667 cm^{-1}, could indicate the presence of aromatic compounds. The peak in the -OH stretch region continues to shift to a higher wavenumber in the day 38 sample, but with the band flattening out, which could suggest degradation of characteristic aliphatic compounds and fatty acids. A shift in the peak around the 1140-1070 cm^{-1} range is observed from day 0 to day 28 to day 38, with what is likely an indication of inorganic compounds becoming more prevalent as the organic compounds are removed from the solution. This could also be an indication of C-N stretching of aliphatic amines or C-O stretch of alcohols and carboxylic acids, although the latter becomes less likely as associated peaks in the range >3000 cm^{-1} become less defined. At day 38, there is the emergence of a peak around 620 cm^{-1}, which could be an indication of halides (Singh *et al.* 2017). There is also an emergence of a strong peak around 1349 cm^{-1} in the day 28 sample, which could indicate methyl groups, which, when in combination with the other peaks identified, and bending around 720 cm^{-1}, could indicate the presence of hydrocarbons. This peak disappears in the day 38 sample, which could indicate degradation, or there could be experimental error, such as inadequate sample concentration of the key organics for detection.

9.4.2.2.2 WWTP_1, 3 and 4_Secondary

The spectra for secondary sludge leachates (Figure 9-22, Figure 9-24, and Figure 9-25) show some similarity to the pattern observed in the primary sludge leachate (Figure 9-21) however, there are some differences. There is a similar shift in the -OH stretch towards a higher wavenumber in the day 28 samples, and this flattens out by day 38 but for WWTP_1 (Figure 9-22) there are more distinct double peaks observed around 2924-2854 cm^{-1} at day 38. This is likely to be C-H stretching of alkyl substances. Again, a shift in the stretches around 1150-1050 cm^{-1} and lengthening of the peak by day 38 is observed indicating inorganic compounds or C-N stretching of aliphatic amines. For day 28 and day 38, the emergence of a peak at around 620 cm^{-1} for WWTP_1 (Figure 9-22) could again indicate the presence of halides. For WWTP_4, (Figure 9-25) there is emergence of the methyl peak around 1350 cm^{-1}, at day 28 that was also seen in day 28 sample for the WWTP_1 primary sludge leachate (Figure 9-21). The combination of peaks in the 2924-2854 cm^{-1} range, the peak and 1350 cm^{-1} and stretch around 1150-1050 cm^{-1} in combination also support the structure of the surfactant LAS (Mottaleb 1999). The differences in the spectra observed for WWTP_3 (Figure 9-24) for day 38 indicates a poor resolution for this sample compared to the day 0 and day 28 samples, preventing detailed analysis of day 38 spectra.

9.4.2.2.3 WWTP_1 Biosolids and Reference Material

The spectra for WWTP_1 biosolid (Figure 9-23) provides maybe the clearest presentation of the shift from day 0 to day 38 leachates of all the samples analysed. This is likely due to the fact that the aqueous leachate also had one of the highest concentrations of DOC, and hence the drying method resulted in a greater amount of residue on the detector compared to some of the other lower concentration samples. This provides some useful insights for future studies of this nature. The pattern observed for the biosolids is similar to that of the other samples, but there are some slight differences. For example, for WWTP_1 (non-nitrifying plant, Figure 9-23), the peaks around 620 cm^{-1}, associated with halides (Singh *et al.* 2017) are present in day 0, day 28 and day 38 samples. In contrast, this only appears as a distinct peak in day 38 samples for the reference material (Figure 9-26), although there is bending in the 680-600 cm^{-1} range for day 0 and day 28, but with less definition. For the reference material (Figure 9-26) there is an absorbance in the 1405-1330 cm^{-1} range, and around 1640 cm^{-1}, both of which could be associated with C-C stretching in aromatic rings in the day 0 sample. This remains in the day 28 and day 38 samples but is reduced in intensity. As with other samples, the day 38 indicates the defined stretch in the 1150-1120 cm^{-1} range indicative of inorganic compounds and C-N stretching of aliphatic amines.

It should be noted that some spectra included an absorbance or transmittance signal at approximately 2360-2340 cm^{-1} that was identified as carbon dioxide, which may have been present as an ambient interference. This peak also appeared for some of the aqueous samples, either as a transmittance or absorbance, but was ignored as potential interference in the spectra.

9.5 Conclusions

This study is the first known experiment comparing UV wavelength scans and FTIR spectral analyses of biosolids and their leachates and biodegradation experiment residuals. The UV wavelength scans provide evidence that the non-biodegradable fraction of biosolid leachates (PDDOC) contain a higher proportion of higher-wavelength compounds (larger, more complex, conjugated compounds) than the DDOC overall. The optical properties of the PDDOC leachates indicate a larger proportion of absorbances at higher wavelength, an increasing SUVA$_{254}$ ratio and a decreasing E2:E3 ratio signalling that the non-biodegradable fraction contains more aromatic, high MW compounds. The study observed lower E2:E3 ratios in the non-nitrifying plant compared to the nitrifying and partially nitrifying plants, which would indicate a greater proportion of high molecular weight compounds in the non-nitrifying plant leachate. The comparison of the SUVA$_{254}$ ratios found that the plant with primary settlement had a lower SUVA$_{254}$ ratio in the secondary sludge compared to plants without primary settlement. This provides an indication that primary settlement removes a significant proportion of high MW, aromatic and hydrophobic compounds. When evaluating the SUVA$_{254}$ ratios for the biosolids however, there appears to be little difference between treatment plants, indicating a similar proportion of aromatic, high MW and hydrophobic compounds, which increases in proportion for all sites from day 0 to day 28. Biosolids are typically comprised of a combination of sludges from across wastewater treatment processes and may include additional inputs from external sources such as septic tanks. The results suggest that combined biosolids, undergoing various types of conventional treatment have a similar proportion of aromatic, high MW and hydrophobic compounds. This suggests that further treatment of biosolids would be needed to reduce concentrations of leachable POPs.

Additional evidence from FTIR scans suggests that remaining persistent material (day 28) may contain functional groups that are consistent with some common persistent pollutants (LAS, and alkyl halides that could include PBDE, PFOS, etc). FTIR also suggests that while AD treatment may not reduce total DOC, there may be a change in the type of compounds present (e.g. metabolites). The PDDOC results from the day 28 and day 38 samples indicated little change in the DOC concentration, however, the FTIR spectra indicate that there may have been some biotransformation of organic substances in the day 38 sample, that was not signalled by the small and variable changes in PDDOC.

The FTIR analyses of dry sludges and biosolids confirm the characteristic spectra that has been observed elsewhere in the literature. All dried solids were observed to present a similar FTIR spectra dominated by aliphatic chains, carbonyl, hydroxyl and amide groups consistent with a range of organic carbon sources including carbohydrates, proteins and fatty acids as the dominant compounds. This study therefore adds to the knowledge base on optical properties of biosolids, for which few published studies exist.

The study finds that biosolid leachates may contain a large number of high molecular weight, highly conjugated and possible halogenated compounds. This suggests that sludges and biosolids may not be completely innocuous when applied to land, with potential transfer of organic pollutants into the environment. This study provides some evidence that additional characterisation of leachates is need before land application of these substances. Further consideration of appropriate end uses for biosolids for the protection of human health and the environment may require further consideration of additional treatment processes that could reduce the POP burden.

The next section discusses the implications of the study results to improved management of biosolids.

CHAPTER 10 IMPLICATIONS OF THE FINDINGS TO REGULATORY FRAMEWORKS

10.1 Introduction

The management of environmental risks associated with land application of biosolids remains a significant challenge for environmental regulators globally. The potential benefits of recycling wastewater solids by application to land are numerous, and include increasing the organic matter content and cation exchange capacity of soils, providing a renewable source of key nutrients (N and P), improving water retention capacity, and offsetting the potential negative impacts that could result from the landfilling (greenhouse gas generating) and disposal at sea as discussed in Chapter 3. However, the literature has demonstrated that wastewater derived biosolids contain a vast number of potentially harmful organic pollutants. This study has also shown that biosolids leach a significant quantity of DOC that can be a carrier of both inorganic and organic pollutants, and that a portion of this DOC contains persistent organic carbon, with indications of high MW compounds, aromatic or halogenated groups. This is consistent with previous studies showing the application of biosolids to land increases the soil concentration of compounds such as PBDEs, PCDD, PCDF, nonylphenols, BPA and other POPs (Wilson *et al*. 1997; Eljarrat *et al*. 2008; Yager *et al*. 2014). More sustainable management solutions are therefore needed by environmental regulators in managing the potential risks of biosolids applied to land. This includes tools that allow better quantification of risk, as well as those that provide an indication of effectiveness of treatment solutions. This chapter reflects on future regulatory considerations for the application of biosolids to land and possible use of the applied methods to establish practical environmental management guidelines for the risk assessment and treatment of municipal wastewater sludges for reducing risks to the environment from POPs.

10.2 Implications of the study results

The review of literature presented in Chapters 2 and 3 highlighted some of the risks to human health and the environment from POPs, and confirms, based on a number of studies, that POPs including PPCPs, industrial chemicals and other organic pollutants are now ubiquitous in municipal wastewater treatment effluent, and increasingly being detected in municipal wastewater treatment solids. These compounds may be present below regulatory limits for individual compounds; however, POPs are rarely present in isolation. This study has indicated that significant quantities of DOC leach from municipal wastewater solids, with between 10,000 and 30,000 $mg \cdot kg^{-1}$ found to leach from samples assessed in this study, as presented in Chapter 7. While much of this DDOC will be innocuous and potentially beneficial DOC for the soil community or crops, it may also be a carrier for some POPs and possibly inorganic pollutants (e.g. trace metals) and a proportion will be persistent compounds. The results from Chapter 8 indicated that between 14-39% of the DOC is persistent (PDDOC) and characterisation of this fraction using bulk analysis techniques of UV-Vis wavelength scanning and FTIR analysis presented in Chapter 9 indicate the presence of POPs in leachates. The comparison has shown that in conventional WWTP, the treatment plant type may affect the DDOC levels resulting from biosolids, however there is less of a notable difference in the PDDOC fraction suggesting that adjustment of operational parameters in conventional treatment processes may not have a significant effect on removal of the most persistent organics. Current wastewater treatment practices to improve the quality of effluents, may lead to concentration of pollutants in sludges, or improve mobility of persistent substances.

The general findings of the research collectively provide evidence that:

- biosolids could present a risk of environmental transfer of POPs;
- leaching tests can be effectively applied to quantify DDOC;
- biodegradation tests can be applied to quantify PDDOC;
- the combined approach provides an alternative bulk quantitative assessment that could be used to estimate pollutant burden or assess the effectiveness of treatment processes.

The next section will examine challenges of existing regulatory frameworks, and how the approach used in this study could be applied.

10.3 Potential application to regulatory frameworks and development of guidelines

10.3.1 Challenges with existing frameworks

To date there have been few regulatory approaches used to control pollutant risks arising from the application of biosolids to land. These are typically limited to controls on pathogens, odour and trace metals, with a few countries providing limits for a small selection of organic compounds as presented in Chapter 3. The pathway risk assessment approach used by the USEPA to quantify risks of biosolids use only considered risks to highly exposed individuals and did not consider legacy effects of cumulative use over time, or the wider environmental implications. The current US regulation under the Part 503 rule, also applied in countries such as Canada, saw organic pollutants removed from consideration due to conclusions that concentrations of individual contaminants were below possible levels of harm, and treatment processes were probably adequate to reduce organic pollutants to acceptable levels. This approach excluded the consideration of compound mixtures, and the cumulative effects of regular application over time, which as shown by other authors leads to a build-up of pollutants in the soil (Eljarrat *et al.* 2008). Some regulatory regimes provide only generic guidelines for organic pollutants in biosolids, specifying that those applying biosolids to land take reasonable actions to reduce harm to health and the environment. However, there is little specification over how this should be achieved, or how risk reduction can be measured.

A paradox appears to exist in the consideration of regulatory regimes for wastewater effluents compared to wastewater solids. Effluents are heavily monitored, and treatment processes are designed to enhance the removal of pollutants to meet quality standards before discharging to the environment. Future regulation of wastewater effluents is likely to include an increasing number of organic compounds. The European Commission for example is expanding the focus on pharmaceuticals and emerging organic pollutants in the environment through the Water Framework Directive (33 priority substances and 8 other compounds). As new standards are introduced and limit concentrations implemented for emerging POPs, wastewater treatment operators will be required to adjust treatment processes to ensure compliance. In contrast, the Sewage Sludge Directive (86/278/EEC) is currently limited to ensuring sludge applied to land has undergone biological, chemical or heat treatment or long-term storage to reduce its fermentability and the health hazards from pathogens, and limit values for seven heavy metals are met (Thomaidi *et al.* 2016). No requirements are placed on plant operators to ensure sludges meet limit values for the wider range of priority substances that are required for liquid effluent, however studies such as this one indicate that sludge leachate may be a pathway for priority substances to reach the environment. In theory, treatment plant operators could design treatments that decrease effluent concentrations by increasing sorption to solids, transferring the pollutant burden to the sludge or biosolid matrix. For example, the use of granular activated carbon as a sorbent is one option for reducing concentrations of pharmaceuticals in effluent. This treatment will not degrade POPs but instead move them into the solid matrix, where they will not be subject to the same regulatory controls as they would be for effluents. Further tools are needed by environmental regulators to assess both the existing risks of environmental contamination from municipal wastewater treatment sludges applied to land and the impact that future regulatory changes to effluent limits could have on pollutant burdens in sludges.

10.3.2 Developing new guidelines

Developing new approaches to managing the environmental and human health risks of applying biosolids to land is needed that consists of two key components: first, the availability of practical tools for assessment of risk, and second, application of risk assessment to regulatory controls and recommended treatments. An example of a recent attempt to do this in the UK was reported by the Scottish Environment Protection Agency (SEPA). SEPA appointed WCA Environment to carry out an assessment of risks to soil quality and human health from organic contaminants in materials commonly spread on land in Scotland in 2014, which included consideration of sewage solids. The approach used standard ERA principles with an adapted RQ calculation based on the quantity of material applied to soil (tonnes per annum) divided by the health criteria value ($\mu g \cdot kg^{-1}$ body weight per day). The process used by WCA Environment is described in Figure 10-1.

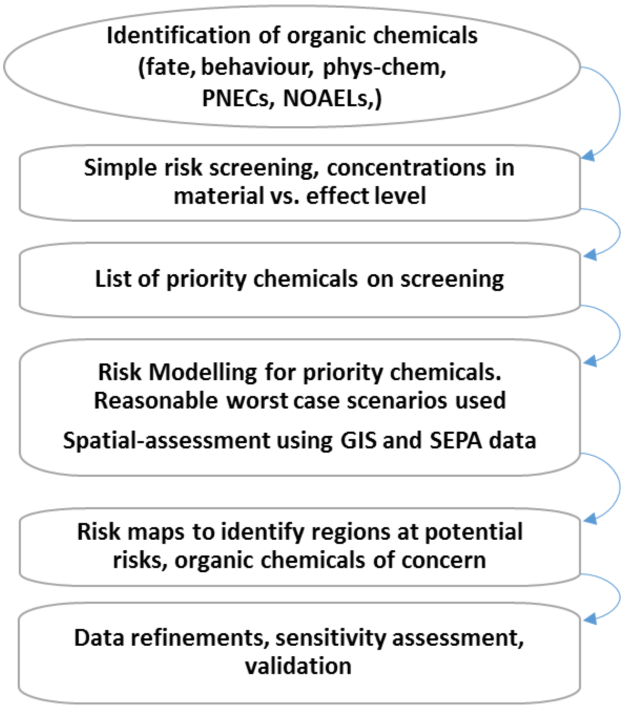


Figure 10-1. Process used by WCA Environment to identify organic contaminants and locations for monitoring of organic contaminants due to land application of materials such as municipal wastewater treatment sludges (Adapted from WCA Environment 2014)

The approach used by WCA applies system-wide thinking in assessing risk such as those discussed by Mansour *et al.* (2016) and Besse and Garric (2008) in Chapter 3 and indicates a progression towards more complexity in risk assessment strategies. Several barriers were encountered in the risk assessment process. Barriers included a lack of local relevant data for predicting environmental concentrations and loading rates for POPs, too many possible pollutants to consider them all, and the reliance on the available literature and published reports for prioritising compounds and quantifying risk. No assessment of mixture effects was carried out. In addition to the risk assessment process, the consultants carried out a survey of laboratories, and their capabilities to perform analytical testing for the priority chemicals identified. The survey found limited national capabilities within commercial laboratories to provide the analytical services required. The process identified a selected number of compounds of interest but has not to date resulted in any new regulatory requirements.

The study carried out by WCA highlights many of the key challenges facing environmental regulators in the management of organic pollutants in general discussed already, namely the vast number of potential analytes and limited availability of practical tools for their monitoring and assessment. In addition, this risk assessment focussed on absolute concentrations in sludges, using solvent extraction techniques that are time consuming and expensive, and may be of limited value to defining risk. These limitations highlight the importance of more practical methods such as the ones applied in this study and leads to consideration of how the concept could be adapted into established regulatory systems.

One approach may be to consider the management of risks associated with biosolid application to land in a manner more closely aligned with contaminated land risk assessment approaches that assess the movement of pollutants from contaminated soil samples. The leaching procedures used in this study apply contaminated land assessment thinking but reverse the concept to assess the level of possible contamination before a material is applied to land rather than assessing the land contamination in-situ. Adapting this approach to use in regulation could reduce existing analytical barriers of assessing whole sludge concentrations of organic pollutants. As an example, the Screening Level Risk Assessment approach used by the Ministry of Environment and Climate Change Strategy in British Columbia Canada applies a leaching procedure to soil samples from land suspected to be contaminated, followed by analysis of a range of possible organic and inorganic contaminants in the leachate (BC MoECC 2019). Applying a concept such as a Screening Level Risk Assessment to biosolids before application to land could increase opportunities for enhanced treatment and reduce the potential for land and water contamination. This approach overcomes the analytical barriers associated with solvent extraction methods for the difficult biosolid matrix, and presents leachates in an aqueous form, more amenable to modern analytical techniques. Many laboratories are already equipped to process these types of samples and have established and accredited methods.

Another consideration in this study is the use of a bulk generic measure (DDOC/PDDOC) to estimate organic compound fluxes from biosolids. This study has provided evidence that these metrics could provide a good general measure of organic compound fluxes and relative proportion of persistent organic carbon, and when coupled with indicative qualitative assessment tools such as UV and FTIR as described in Chapter 9 provide some qualitative analysis. This reduces barriers such as cost and inadequate analytical capacity and reduces the need to prioritise individual pollutants for analysis, allowing for an indication of the total composition of a compound mixture that is present. The proposed simplified approach is set out in Figure 10-2.

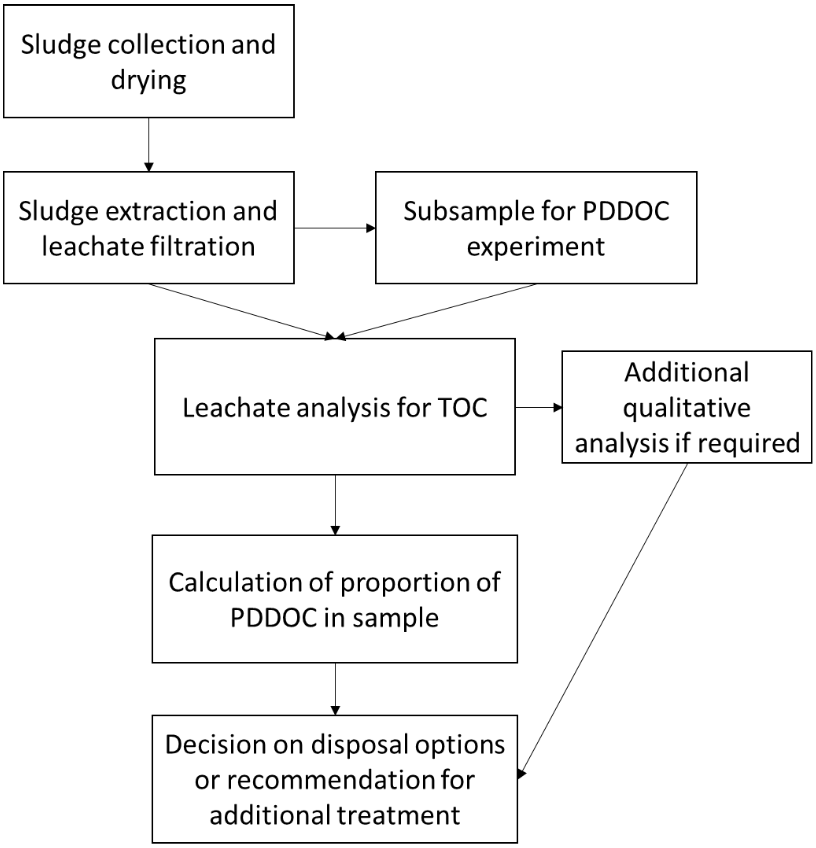


Figure 10-2 Risk assessment approach for PDDOC in biosolids

10.3.3 Limitations of the approach

There are some limitations to the proposed approach. First, while the PDDOC approach allows for quantitative assessment of persistence of a compound mixture, there is no characterisation of the precise identity and relative quantity of organic substances leaching from biosolids. This may limit the ability for regulators to declare, confidently, that a specific PDDOC level is also associated with a specific level of possible harm. This could be overcome by establishing correlations between PDDOC and toxicity over a wide range of biosolids. Leachate based toxicity tests can be carried out to assess the toxic effects of an unknown chemical mixture on various target organisms in the form it is released into the environment. This method doesn't require specific knowledge of the chemical composition and overcomes the barrier of unknown physicochemical interactions that can occur between contaminants within a mixture (Backhaus *et al.* 2003). Assessment of toxicity of mixtures was outside the scope of this study but could be a valuable area for further research.

Secondly, the PDDOC experiments used in this study were performed over 28 d, which may not be a practical time frame for quick decisions on application to land. While this study used 28 d as the norm used in biodegradation experiments, most of the biological removal occurred within the first two days and was virtually stable within seven days. Therefore, a condensed biodegradation phase could be considered. In addition, the use of the DDOC test alone, without the biodegradation step provides a valuable metric for assessing DDOC fluxes. DDOC coupled with the additional qualitative analysis tools could provide useful information on the quality of the sludge matrix without carrying out additional biodegradation stages.

10.4 Potential development of sludge treatment guidelines

The approach, if adopted in the proposed format or adapted to assess leachates in another manner, would require practical advice to treat biosolids that were found to exceed regulatory limits. The primary options available to treatment plant operators are discussed in this section.

10.4.1 Source control

Sludge treatment and management already makes up a major proportion of WWTP costs ranging from 18-57% of total wastewater treatment costs (LeBlanc *et al.* 2008). Operators will understandably be hesitant to adopt additional sludge treatment options if they are not legally obligated to do so. Therefore, measures that can reduce the inputs of POPs into wastewater treatment systems at source could be an appropriate management consideration. This may only be possible for some types of compounds, for example industrial or hospital sources of organic compounds that can be treated onsite by the industrial customers. Using a PDDOC reduction metric in regulatory discharge consents could be considered for these large effluent producers to encourage reduction of PDDOC at source.

For many PPCPs however, use is typically within the home and there is limited ability to reduce these materials entering sewers other than to discourage the public from disposing of expired or unneeded pharmaceuticals and household chemicals in household sinks or toilets. Working with producers to develop greener products that contain fewer POPs could also be considered as a long-term measure. With public awareness of substances such as micro-plastics in the water system increasing, producers have begun to phase out use of microbeads in products such as cosmetics and toothpastes and consumers are becoming more aware of the connection between wastewater and the environment, which is promising for future source control measures.

10.4.2 Sludge treatment

As demonstrated in this study, process parameters and treatment type appear to influence the overall DDOC concentrations observed. Nitrifying plants are found to have lower DDOC, as are biosolids that have undergone additional treatment such as thermal treatment as observed for the reference material and AD treatment observed for the biosolids from WWTP_1. If the overall goal is to reduce DDOC, then standard approaches used in treatment works to reduce BOD and organic matter also appear to reduce DDOC leached from sludges. If the goal is to reduce PDDOC however, further considerations are required.

The results of this study demonstrated that although differences were observed in the PDDOC of treatment plants operated under different conditions, most biosolids still contained an appreciable concentration suggesting that additional treatment such as nitrification, AD and thermal treatment may only reduce DDOC significantly and result in substrates with more concentrated PDDOC. This may appear counter intuitive that plants with which have been shown to reduce some POPs in effluent, are not necessarily effective in reducing concentrations in sludges. Some of the extended treatment may reduce readily biodegradable substances, leaving the highly non-biodegradable substances behind, or providing more time for sorption processes to occur as discussed in Chapter 2. Further solutions are therefore needed for reduction of PDDOC. Potential treatments include those that enhance biological or chemical degradation processes. Common treatments are discussed in the following sections.

10.4.2.1 Aerobic digestion and composting

There is some evidence that additional aerobic treatment could be effective in reducing some organic compounds in sludge. Aeration of thickened sludge can enhance volatilisation of some compounds and enhance conditions for biological breakdown and has been shown to result in some additional degradation of some persistent compounds such as hormones, nonylphenols and PAHs (Semblante *et al.* 2015). Composting processes may be more effective than aeration alone, but effectiveness can be dependent on the type of bulking agent (e.g. wood chip, green waste etc.). For highly resistant compounds, aeration and composting may have limited effect. To increase effectiveness, pre-treatment of sludges using techniques such as advanced oxidation or enhanced biological treatment may be considered as discussed below.

10.4.2.2 Anaerobic digestion (AD)

As found in this study, additional treatment of biosolids by AD may have limited impact on PDDOC. Although there are greater sustainability benefits due to potential energy generation from AD processes, there is generally poor reduction in persistent compounds in typical mesophilic or thermophilic processes (Semblante *et al.* 2015). Pre-treatment of sludges for both aerobic and AD processes may increase the bioavailability of some compounds by causing desorption from sludges and therefore pre-treatments such as oxidation processes may be coupled with conventional AD treatments to enhance removal.

10.4.2.3 Advanced oxidation

Advanced oxidation processes (AOP) have been widely studied for their effectiveness at degrading organic compounds. These can be applied in a range of configurations and can either be used as a pre-treatment or direct treatment (Carballa *et al.* 2007a). AOP using ozone treatment has been found to provide effective removal of >70% for many pharmaceuticals, hormones, and phthalates in municipal wastewater (Choubert 2016). Magureanu *et al.* (2015) reviewed the literature on effectiveness of various advanced oxidation processes (AOPs) on pharmaceuticals in wastewater, specifically diclofenac, and found good removal rates for most techniques as summarised in Table 10-1. Semblante *et al.* (2015) also reviewed the literature on effectiveness of AOP on removal of organic compounds including PAHs and various hormones and pharmaceuticals from wastewater sludges (Table 10-1). Effectiveness varied based on the compound type, and how the treatment was applied (e.g. pre-treatment of anaerobic digester sludge, or post-treatment of sludges or digestate). While these processes demonstrated high removal efficiencies for some compounds, their application can include additional operational and cost considerations due to energy and chemical requirements. Magureanu *et al.* (2015) also note that while AOPs are effective in some parent compound removal, degradation products may be less readily removed, and some may be potentially more harmful.

Table 10-1 Degradation rates of diclofenac by various advanced oxidation processes reported in the literature (Adapted from Magureanu *et al.* 2015 and Semblante *et al.* 2015)

	Removal rate (%)			
	Diclofenac	PAHs	Pharmaceuticals and hormones	Other compounds
Process type				
Non-thermal plasma	100			
Ozonation	>99 to 100	50-60	Enhanced removal some hormones	Enhanced removal for carbamazepine, less impact for BPA and nonylphenol
Ozonation + TiO_2 photocatalysis	100			
TiO_2 photocatalysis	85 to >95	21-63		
Sonolysis/ultrasonication	>90		Increased aqueous concentrations in aerobic reactor	Increased naphthalene removal to 54-65%, improved removal of phthalate esters
Sonolysis + TiO_2 catalysis	84			
Peroxide (H_2O_2) + UV	>95		67-97 removal of BPA and various hormones	Enhanced carbamazepine removal to 45%
UV oxidation		15		
Photo-Fenton (solar light plus H_2O_2/Fe)	100	30-67	70-98	phthalates improved removal to 85%
Reference	(Magureanu *et al.* 2015)	(Semblante *et al.* 2015)	(Semblante *et al.* 2015)	(Semblante *et al.* 2015)

10.4.2.4 Enhanced biological processes

Biological processes including bioaugmentation (addition of specialised biological agents) or bio-stimulation (enhanced conditions for stimulating growth and activity of degrading organisms) have been considered for enhanced removal of organic compounds. Bioaugmentation has included the use of exogenous assemblages of either bacteria, yeasts or fungi mixed with sludges to enhance degradation processes. These processes have been found to be effective for some high molecular weight compounds, however effectiveness varies by the type of bioaugmentation and the compounds present (Semrany *et al.* 2012). Fungal treatments have shown some promising results for effective removal of compounds such as estrogens but have been found to show mixed results for other compounds such as brominated flame retardants and varying removal efficiencies ranging from 40 to 100% across some pharmaceuticals (Vincent *et al.* 2016). The review by Semblante *et al.* (2015) found similar results for treatment by white rot fungi (WRF), however noted that WRF may be effective for reducing some organic compounds for which bacterial augmentation was found to be ineffective. In the review by Raper *et al.* (2018) the use of bioaugmentation and bio-stimulation to remove thiocyanate ($SCN^–$), PAHs, phenol and trace metals in coke wastewater was investigated. An adapted microbial consortia of *Bacillus sp*. was found to increase removal of PAHs to compliant levels for effluent. The study also assessed the use of bio-stimulation through additions of micronutrients and alkalinity to enhance bacterial growth. This resulted in reductions in SCN^- and phenol.

Another type of biological process, bioleaching, has been widely applied for remediation of metals in contaminated soils and other matrices, but has only recently been studied for application to the removal of organic substances. Bioleaching involves the conditioning of sludge with bacteria such as *Acidithiobacillus ferrooxidans* to assist in dewatering of sludges before further treatment. Lu *et al.* (2019) found that using bioleaching as a dewatering technique increased the bioavailability of PAHs in dewatered sludge and enhanced their subsequent removal during composting processes. This same effect was not observed using chemical de-watering methods such as addition of polyacrylamide or Fe[III]/CaO.

Enhanced biological processes may require additional study to assess effectiveness on removal of specific compounds but have the benefit of being more environmentally friendly and cost effective compared to advanced oxidation processes (Semblante *et al.* 2015). Effectiveness of biological processes for sludges may be limited on the ability of the biological community to oxidise and/or hydrolyse organic compounds, for example through the production of enzymes, rather that consumption and incorporation into intracellular material. PDDOC could provide a suitable indicator to assess the efficacy of some of these treatments on reducing POP concentrations in biosolids.

10.4.2.5 High temperature thermal treatment

A final option for treatment of biosolids is high temperature thermal treatment such as incineration or pyrolysis. Incineration is a preferred option in many countries as described in Chapter 3. This option provides the opportunity to treat sludge and generate energy, potentially in co-incineration with other types of waste, at a very high temperature that can degrade a large number of persistent pollutants. However, it will not degrade all POPs, particularly some emerging compounds. For example, PBDEs are brominated flame retardants and are designed to be thermally resistant (UNEP 2014). Negative impacts of incineration can include the potential to produce harmful emissions and the generation of incinerator ash, which is typically treated as hazardous waste. Additional formation of PCDDs/PCDFs can be a by-product of incineration of organic material, particularly where chlorinated compounds are present. An option to improve the sustainability of the process is to treat gaseous emissions using activated carbon filters to remove these compounds, and to reuse incinerator ash in building and construction materials (Hong *et al*. 2013). Other thermal treatments such as pyrolysis, which produce biochars, are also considered but there have been few studies assessing the pollutant transfer from biosolid derived biochars into the environment. This could be an area for further exploration in the future (Antunes *et al*. 2017).

10.5 Conclusions

This chapter has proposed that the results of this study could be used to develop a new approach to environmental risk assessment of organic contaminant transfer from biosolids applied to land, using an approach similar to the screening risk assessment applied to contaminated land sites elsewhere. This approach may assist decision makers by simplifying the analytical requirements and providing a generic bulk measurement that could be used for screening biosolids before land application. The approach could be refined by considering leachate toxicity and streamlining biodegradation steps to improve relevance and practicality of PDDOC measures. With an approach such as this, regulators could then assist sludge producers in applying effective and sustainable treatment solutions, with AOP and enhanced biological processes being the most promising options for future consideration. Subsequent PDDOC testing could be used to confirm effectiveness of removal of recalcitrant compounds, but similarly, UV wavelength scanning may provide a quicker comparison of relative reduction. These approaches, however, are unlikely to be considered while legislation for control of organic pollutants in biosolids remains absent.

CHAPTER 11 CONCLUSION

This chapter summarises the results and key findings of the study. This section also discusses the implications and recommendations arising from the research and future directions of study.

11.1 Summary of the results and discussion

11.1.1 Research conducted

The overall aim of the work was to investigate the desorbability and persistence of dissolved organic carbon in various municipal WWTP sludges and biosolids. This work was conducted to improve the understanding of persistent organic carbon that may migrate from biosolids into the environment, and to investigate the impact of wastewater treatment processes on its composition. This study has provided a new approach to investigating the risk of persistent organic pollutant transfer from biosolids applied to land and presented a new tool for assessing effectiveness of treatment processes on reduction of persistent organic pollutants in sludges and biosolids.

The work has overcome several existing barriers to effective assessment and management of the organic pollutant risks associated with wastewater treatment derived biosolids with development of an appropriate extraction, biodegradation and analysis protocol. The first stage of the study used leaching techniques to assess the migration of desorbable dissolved organic carbon (DDOC) from municipal wastewater treatment sludges and biosolids. The second stage of the research used biodegradation tests to quantify the relative proportion of the DDOC that was persistent (PDDOC). The third stage of the research investigated the optical properties of the DDOC and PDDOC leachates using UV-Vis and FTIR techniques. The results of these three stages of study, and additional consideration of environmental risk assessment practices were used to recommend improved risk assessment approaches for biosolids applied to land, and incorporation into practical environmental guidelines for treatment and handling of wastewater sludges and biosolids.

11.1.2 The key findings from the research

11.1.2.1 Assessing organic carbon mobility

A review of commonly applied leaching tests and a study to assess the effect of pH and shaking time on biosolids extraction was used to confirm the appropriateness of the adopted methodology and to provide insight for future application of leaching procedures on biosolids. The study confirmed that biosolid DOC can be effectively extracted using the test conditions specified in a similar manner to soils, with no notable barriers specific to the biosolid matrix. The effect of pH was found to be of little impact on the overall extraction process for biosolids due to the high buffer capacity, likely due to pH adjustment and lime stabilisation used in most WWTP. While leachate studies used for contaminated land assessment often prescribe an acidic pH, it was found that starting pH for biosolid leaching tests may be less important, therefore future leaching protocols may find that use of neutral water is sufficient for these types of samples, by-passing a sample preparation step and eliminating a potential source of interference and contamination. The assessment of shaking time found that the parameters used were suitable given the alignment with most leaching tests (18-24 h) and results indicated that a reduced leaching time (e.g. 4 or 8 h) underestimated the leachable DDOC.

The leaching tests performed for the various treatment stages and plant types found that there was some variation in results depending on the treatment stage and final treatment type. A low level of variability in DDOC was observed between leachates from primary treatment sludges, where DDOC ranged from 21,692-26,537 $mg \cdot kg^{-1}$, compared to leachates from secondary treatment sludges, whose DDOC varied by the type of treatment plant. DDOC ranged from 11,760 $mg \cdot kg^{-1}$ in a nitrifying treatment system to 33,853 $mg \cdot kg^{-1}$ in a non-nitrifying treatment system. The results indicated that extended aeration, characteristic of nitrifying treatment plants, may have a positive impact on the reduction of DDOC in the secondary sludge samples.

Sludge treatment was found to produce varying levels of DDOC in treated biosolids, which varied from 14,422 $mg \cdot kg^{-1}$ for the thermally treated reference material to 22,542-27,862 $mg \cdot kg^{-1}$ for anaerobically treated biosolids to 26,155-29,983 $mg \cdot kg^{-1}$ for dewatered sludges. The findings suggest that sludge treatment can reduce total

DDOC content with thermal treatment having the greatest impact followed by AD and dewatering processes.

A study of the relationship between DDOC in leachates and trace metals (Cd and Pb) was carried out, with results found to be somewhat inconclusive. Cd was found to be below detection limits for all samples measured. For leachates with detectable Pb concentrations, DDOC was found to be negatively correlated, with the samples with the highest Pb concentrations also having the lowest DDOC. The results are similar to others found in the literature that suggest a higher total organic matter content may be associated with more sorption potential, and less desorption of Pb. The correlation between DDOC and Pb, however, was found to be insignificant when a single high value was removed; therefore, the conclusions that can be drawn from this study are limited. The study does, however, suggest that further investigation of DDOC and trace metal fluxes from biosolids may be useful for understanding the effect of DOC on inorganic contaminants. Further study should include examination of leachates and solids (for total lead and total carbon) to better understand the relative partitioning and leaching behaviour of DDOC and metals. In addition, a wider suite of trace metals could be assessed, using techniques with lower levels of detection, capable of detecting trace concentrations.

The general findings of the leaching study are as expected based on literature, that is, additional processing through enhanced biological or thermal treatment reduces total organic carbon in sludges, and hence reduces DDOC. There are few studies however that have assessed DOC in a biosolids leaching context, therefore this represents one of the first studies that explicitly quantifies leachable DOC in biosolids. Further study on additional plants, operated under varying conditions and a larger number of samples could be useful in confirming the applicability of these findings more widely.

11.1.2.2 Assessing organic carbon persistence

The relative quantity of persistent DOC in leachates was determined using standard aerobic biodegradability tests. Biodegradation profiles showed a similar pattern of DOC biodegradation for all sludges and biosolids samples assessed in the study. Most biodegradation occurred within the first two days of the study, with maximum biodegradation achieved by day 7 for most samples. Future biodegradation studies could consider whether a full 28 day study is required. Although maximum biodegradation appeared to be achieved well within this time frame, it is unknown whether transformation processes were continuing to occur in the leachates. Further study could consider carrying out UV-wavelength scans at interim points in the biodegradation process, to further characterise the degradation process.

The quantity of PDDOC measured in sludges and biosolids ranged from 4,096 $mg{\cdot}kg^{-1}$ in secondary sludge from a partially-nitrifying plant to 7,547 of $mg{\cdot}kg^{-1}$ in a treated biosolid from a non-nitrifying plant. The levels of PDDOC were generally higher in biosolids that had undergone final treatment as compared to sludges obtained from primary or secondary treatment stages. Tests for statistical difference found that primary and secondary sludges had lower mean PDDOC than biosolids. Secondary treatment sludges from the nitrifying plant were found to have lower PDDOC than sludges from the partially-nitrifying and non-nitrifying plants. These results suggest that post treatment of biosolids concentrates persistent organic carbon or increases the desorption potential or mobility of persistent compounds. The results also suggest that extended aeration processes used in nitrifying plants may provide some additional removal of persistent compounds. Although significant, these differences were of a small magnitude, and further study across a larger number of systems and sludge samples could be undertaken to confirm the observed effect.

The additional anaerobic biodegradation study detected little to no additional effect on PDDOC reduction, which agrees with results found in the literature on the use of anaerobic processes to degrade persistent organic carbon, which find limited effectiveness. The test protocol contains some areas of uncertainty related to the selected inoculum and the short 10 day time scale for the anaerobic study, which may have been insufficient for the inoculum to acclimatise to the leachate content. Further study could be conducted to assess whether a better adapted inoculum or a longer adaptation phase may increase PDDOC removal.

11.1.2.3 Qualitative analysis

The qualitative analysis of optical properties of the DDOC and PDDOC leachates was used to identify relationships between DOC and indicators of persistent organic pollutants. The application of optical property analysis has only been applied to biosolids in a limited number of examples in the literature but offers a potentially useful mechanism to assess the quality of biosolids and leachates without the need for expensive and time-consuming analytical methods.

UV wavelength scans provided a visual presentation of the biodegradation process, demonstrating a reduction in absorbance at wavelengths associated with proteins, carbohydrates and fatty acids amongst other biodegradable compounds following the biodegradation study. The study also clearly showed almost complete non-removal of compounds at higher wavelengths (e.g. >300 nm), consistent with more complex, conjugated organic compounds. Further examination of the UV outputs found that calculation of the absorbance ratio E2:E3 and $SUVA_{254}$ both indicated an increase in the proportion of high molecular weight compounds and aromatic compounds after the biodegradation experiment for all sludges and biosolid types. The comparison of treatment plant type found that the non-nitrifying plants indicated a higher proportion of high molecular weight, aromatic compounds than the nitrifying and partially-nitrifying plants, confirming the positive effect of extended aeration and aerobic treatment on reduction of substances such as PAHs. The results also indicate that treated biosolids had a higher proportion of high molecular weight, aromatic compounds compared to sludges obtained from primary or secondary treatment stages. This confirms that sludge treatment results in a concentration of more persistent compounds in biosolid leachates. Another finding from the examination of $SUVA_{254}$ is that primary settlement can reduce the proportion of high molecular weight, aromatic compounds that would otherwise accumulate in secondary treatment sludges. In plants without primary treatment, $SUVA_{254}$ was higher in the secondary sludge leachates, even for plants with extended aeration. This suggests that primary treatment can reduce some of the pollutant burden in subsequent sludge matrices, however in most treatment plants, sludges from all stages of treatment are bulked together. Further study could assess whether additional advanced sludge treatment of primary sludge only, prior to bulking with other sludges, could have a beneficial effect on reducing the organic pollutant burden on the combined biosolids produced in a treatment plant.

The FTIR scanning of both solids and leachates was carried out to provide additional characterisation of the biosolids, and to examine changes in dominant functional groups present in DDOC and PDDOC leachates. The analysis of solid samples identified a characteristic FTIR profile that is similar to other limited examples found in the literature. This provides confirmation of some of the dominant functional groups across all the biosolids that are consistent with the presence of aliphatic chains with double bonds as well as readily biodegradable compounds including fatty acids, carbohydrates and proteins, and indications of inorganic minerals.

The FTIR scans of leachates following biodegradation experiments contain functional groups that are consistent with some common persistent pollutants (e.g. halogenated organics such as PBDE, PFOS). Although little difference in DOC levels were found in leachates from the aerobic and anaerobic experiments, the FTIR spectra of leachates from the anaerobic experiment indicated distinct differences from those following the aerobic study. This suggests that some biotransformation of dominant organic carbon compounds in the anaerobic study may have occurred, but transformation may have been to other persistent by-products not detectable using DOC measurements. Further study could be carried out on more concentrated samples to confirm the findings in this study.

11.1.2.4 Potential application of the results to development of practical environmental management guidelines

Consideration of future directions for environmental regulation was presented in Chapter 10 to examine how the results can be used to inform practical environmental management guidelines for the assessment and treatment of municipal wastewater sludges to reduce risks of transfer of POPs to the environment. The method applied in this study is novel in that it bypasses many of the cost barriers and analytical challenges of measuring individual concentrations of a large number of contaminants in biosolids. It also provides the opportunity to assess a compound mixture and provide a bulk measurement of the persistent organic carbon fraction in a biosolid, which can be used in an approach such as a screening risk assessment similar to that used for assessment of contaminated land. This approach can also be used to measure the effectiveness of various treatment solutions, which to date have typically only been applied to individual or small groups of compounds. The literature clearly identifies that various treatment technologies are promising for some types of compounds, but wide variability in effectiveness is observed for different contaminants. The PDDOC metric provides a useful measurement for rating overall effectiveness of a range of treatment technologies for reducing POPs in biosolids before application to land. Some of the existing limitations of this method include the lack of correlation between PDDOC and toxicity, which could be overcome through further study of the toxicity effects of biosolid leachates.

11.2 General conclusions from the research

The study has provided a baseline of the desorbable dissolved organic carbon likely to transfer from municipal wastewater treatment sludges and biosolids under leaching conditions and has provided an estimate of the quantity of DOC that is likely to be persistent. From the review of the literature, this is the first study of this type, measuring leachable DOC from biosolids coupled with biodegradation tests and comparing leachates across treatment plant types and stages of treatment. The results provide greater understanding of a key characteristic of biosolids (DDOC and PDDOC) that is of direct relevance to the transfer of organic micropollutants into the environment.

The overall results of this study suggest that current WWTP processes and sludge treatment may not be sufficient to reduce POP loads in biosolids to reduce the risk of organic pollutant transfer from biosolids applied to land. Significant quantities of persistent organic carbon remain in biosolids, and despite the common perception that most of this persistent material is tightly sorbed to solids and immobile, this study has demonstrated that some persistent organic carbon is also mobile. Concentrations of persistent mobile organic carbon in the region of 5,000 $mg{\cdot}kg^{-1}$ warrants further consideration by environmental regulators on the potential risk associated with land application. Analysis of optical properties of sludge leachates also indicates that the persistent fraction of leachate contains a relatively higher proportion of high molecular weight, aromatic compounds and evidence of chemical compounds of concern. The results also show that WWTP that apply biological treatment, such as extended aeration for nitrification and sludge treatment, such as thermal treatment or AD, may produce biosolids with an overall reduced proportion of DDOC but concentrated proportion of PDDOC.

The methodology applied in this study provides an example of a low-cost and readily accessible method for assessing the organic pollutant transfer potential from biosolids. This provides environmental regulators and producers of biosolids, or other potentially contaminated waste materials, a useful tool for assisting decision making on further treatment or disposal. One of the main barriers to current regulation is the lack of practical measurements that can be used to assess the overall effectiveness of advanced treatments. The literature indicates that POP removal is variable across treatment types and the interventions required to adapt treatment systems for removal of emerging compounds is of limited consideration for most operators unless operating under a specific regulatory consent condition. As demonstrated in the literature, treatment processes vary in their effectiveness across different types of compounds under differing conditions and therefore it is difficult to define suitable reference compounds to assess process treatment effectiveness. The use of PDDOC as a generic bulk-quantification method helps to overcome this barrier. Experimental procedures that improve the understanding of pathways of exposure, such as movement of organic pollutants from solid to aqueous compartments are needed. The use of comparable and repeatable methodologies for estimating DDOC fluxes from biosolids can improve understanding of the impacts of DOC in the movement of organic micropollutants from wastewater treatment solids. This study has provided:

- Analytical measurements that bypass the need to measure and monitor all possible compounds and can better estimate the total pollutant burden, where mixtures of compounds, metabolites and transformation by-products exist (non-targeted qualitative screening).
- Experimental procedures that characterise the total organic component within biosolids (e.g. biodegradability) derived from various treatment systems to inform wastewater treatment processes to better manage organic pollutant risks.

This study faced some limitations of analytical methods that are outlined in the relevant chapters. Notably, leaching techniques could be limited by the uniformity of sample particles and particle size, and further study could be carried out to assess the effect of particle size. Biodegradation tests may have been limited by the choice of inoculum in the aerobic study, and the length of adaptation phase for the microbial consortia in the anaerobic study. In addition, the use of techniques such as UV-Vis and FTIR analysis provide broad general indicators of the types of compounds, or chemical structures present in a mixture but do not provide precise identification of specific compounds, limiting the ability to state conclusively which POPs may be present. The study was limited by the fixed laboratory conditions of pH and temperature, which may be less likely to represent fluctuating environmental conditions, or application to different environments such as tropical countries. The study was also limited to a small number of available functional wastewater treatment plants for sample collection. Further work could be carried out with a wider range of plant types and different operating conditions. The effects of temperature could also be evaluated by assessing plants in winter and summer conditions, and plants operating in tropical climates. In addition, further work could be carried out to assess the impact of DDOC on movement of inorganic compounds from sludges. This could include a wider range of trace metals assessed at both total and leachable concentrations.

11.3 Future perspectives

In spite of the difficulties with regulating persistent organic pollutants in biosolids, growing awareness of the ubiquitous nature of many organic compounds including pharmaceuticals in the environment is causing environmental regulators to reconsider approaches to management of municipal wastewater treatment by-products. Movement towards increasing the number of organic pollutants in effluents are already being made in many countries aligned to new additions to the SCPOP however regulation for land disposal of biosolids has not kept pace, with much of the current regulation out of date, thereby signalling unwillingness amongst some regulators to address this potential source of environmental contaminants. Changes to wastewater processing for removal of new priority pollutants is likely to impact on pollutant concentrations in sludges. There is a growing lobby amongst wastewater treatment operators to increase the land application of biosolids for beneficial uses such as agriculture and land reclamation, and new initiatives such as the biosolids assurance scheme recently launched in the UK is evidence of this. These two changing practices highlight the need for environmental regulators to have practical tools at their disposal to evaluate risk and provide guidance to operators.

Very few jurisdictions around the world have regulatory protocols in place to limit organic pollutant concentrations in biosolids spread to land. The combination of the quantitative and qualitative techniques described in this study provides one potential approach to assessing environmental risk from the transfer of organic compounds from land application of biosolids. The implications of the study to assess DDOC and PDDOC could also be used beyond the scope of biosolids, in addressing organic pollutant transfer from other types of waste materials disposed of to land. The generic measure could also be used to evaluate contaminated soils or other substances before disposal.

11.3.1 Directions for further research

As with any experiment, additional study using the same experimental parameters could provide additional evidence to assess the applicability of the approach to sludge management practices. This could be expanded to assess a wider range of treatment processes such as membrane bioreactors (MBR), which have not been considered in this study, but are an increasingly important type of wastewater treatment process. More work is needed to characterise and examine the influence of common sludge treatment techniques on biosolids (e.g. thermal, advanced oxidation etc.) The literature suggests that these treatments and the use of MBR may be effective in reducing the POP levels in sludges. Further study is therefore required to assess their impacts on the levels of PDDOC.

Future investigations could include additional leaching studies on a wider range of sludge types and could also expand to consider other types of waste materials, or contaminated soils to assess the effectiveness of the methodology on non-sludge matrices. Future study on the toxicity of biosolid leachates could be conducted to assess whether correlations exist between PDDOC and various toxicity endpoints including acute and chronic effects. This could help to validate the parameter as an appropriate measure for use in environmental monitoring or regulation. On a similar note, additional characterisation of biosolid leachates could be conducted to analyse leachates for a selected number of key compounds to identify if correlations exist between PDDOC and compounds such as PAHs, PBDEs, PFOS, triclosan, or LAS.

Chapter 12 Appendices

Appendix 1 Sample collection details

Table 12-1 Details of sludge collection

Plant ID	Date and Time of collection	Sampling details (weather and observations)
WWTP_1 (Apr)	12/04/17; 09:00	12°C, Weather mixed cloud and sun, dry, preceding week was similar. Sludge collected from primary settlement tank, secondary settlement tank (pre-chemical addition). Dry pellets were also collected from bulk bags. Collected by site operative.
WWTP_1 (May)	08/05/17; 09:30	12 °C, Dry weather (no rain since previous sampling event). Sludge collected from primary settlement tank, secondary settlement tank (pre-chemical addition). Collected by site operative. An older sample of sludge pellets (>1 yr) was obtained
WWTP_2	05/04/17; 11:30	11°C, Weather cloudy and dry, preceding week was similar. Sludge collected from primary settlement tank and oxidation ditch. Collected by site operative.
WWTP_3	12/04/17; 13:30	14°C, Weather mixed cloud and sun, dry, preceding week was similar. Sludge collected from secondary activated sludge tank (pre-chemical addition). Dewatered sludge cake was also collected. Sludge was collected directly from the activate sludge tank by the primary researcher in this study, and sludge cake was collected from bulk containers by the site operative.
WWTP_4	16/05/17; 11:00	17°C, Weather dry, warm and cloudy. The day prior to sampling was first substantial rain in weeks. Sludge collected from SBR tank by the site operative into buckets, then transferred to sample bottles by the primary researcher in this study.

Appendix 2 Calculation of moisture content

Calculation of % Moisture in sludge samples

Date	Treatment plant	Sample Name/ Replicate number	Crucible mass (g)	Crucible + sample (g)	Crucible + sample after 24 h at 105°C (g)	Wet weight of sample (g)	Dry weight of sample (g)	% moisture in sample	% moisture (average)	% difference between reps
30/05/17	1	Biosolid/A1	19.19	20.190	20.125	1.000	0.935	6.50%	6.56%	2%
30/05/17	1	Biosolid/A2	19.89	20.887	20.821	0.997	0.931	6.62%		
30/05/17	1	Biosolid/B1	18.77	19.772	19.721	1.002	0.951	5.09%	5.17%	3%
30/05/17	1	Biosolid B2	19.68	20.691	20.638	1.011	0.958	5.24%		
02/06/17	1	Primary/A1	19.32	20.328	20.209	1.008	0.889	11.81%	11.66%	2%
02/06/17	1	Primary/A2	19.56	20.567	20.451	1.007	0.891	11.52%		
02/06/17	1	Secondary/ A1	16.98	17.985	17.856	1.005	0.876	12.84%	13.10%	4%
02/06/17	1	Secondary/ A2	18.63	19.632	19.498	1.002	0.868	13.37%		
07/06/17	1	Primary/B1	19.676	20.6704	20.5678	0.994	0.892	10.32%	10.12%	4%
07/06/17	1	Primary B2	19.189	20.1854	20.0866	0.996	0.898	9.92%		
07/06/17	1	Secondary B1	20.893	21.8900	21.7828	0.997	0.890	10.75%	10.75%	*
12/06/17	2	Primary/1	20.888	21.8857	21.8109	0.998	0.923	7.50%	7.50%	*
12/06/17	2	Secondary/1	19.187	20.1887	20.0675	1.002	0.880	12.10%	12.10%	*
21/06/17	3	Secondary/1	19.325	20.3241	20.192	0.999	0.867	13.22%	13.21%	0%
21/06/17	3	Secondary/2	19.676	20.6861	20.5527	1.010	0.877	13.21%		
21/06/17	3	Biosolid/1	19.563	20.5804	20.443	1.017	0.880	13.51%	13.58%	1%
21/06/17	3	Biosolid/2	20.892	21.9120	21.7728	1.020	0.881	13.65%		
27/06/17	4	Secondary/1	19.325	20.3245	20.1999	1.000	0.875	12.47%	12.54%	1%
27/06/17	4	Secondary/2	19.565	20.5629	20.437	0.998	0.872	12.62%		
27/02/18	4	Biosolid/1	19.4315	20.4002	20.2782	0.9687	0.8467	14.4%	14.36%	1%
27/02/18	4	Biosolid/2	16.6315	17.5157	17.405	0.8842	0.7735	14.3%		
03/07/17	7	Reference	18.6332	19.6313	19.497	0.998	0.864	13.46%	13.46%	*

*single replicate due to limited sample mass available

Appendix 3 Calculation of volatile solids

Volatile solids (VS) were calculated as a percentage of material lost on ignition at 550 °C as a percentage of sample dry weight using the following calculation:

$$\% \text{ Volatile solids (VS)} = \left(1 - \frac{final\ weight\ after\ ignition}{final\ weight\ after\ drying\ at\ 105°C}\right) \times 100$$

Raw data and calculated V_S are presented in Table 12-2, and Figure 12.1.

Table 12-2 Data and calculated volatile solids for sludge samples

Site ID	Crucible (g)	Crucible plus sample (g)	Crucible plus sample after drying at 105°C for 24 h (g)	Dry Weight (105°C) (g)	Crucible plus sample after ignition 550°C (g)	Non-volatile weight (g)	% Volatile solids (VS)
WWTP_1_BS_old	19.674	20.867	20.779	1.105	20.0695	0.3955	64.2%
WWTP_1_BS_old	18.6302	19.7091	19.6281	0.9979	18.985	0.3548	64.4%
WWTP_1_Pri_A	27.1062	28.0837	27.9868	0.8806	27.4317	0.3255	63.0%
WWTP_1_Pri_B	18.8158	19.7261	19.6394	0.8236	19.0913	0.2755	66.5%
WWTP_1_Sec_A	10.8712	11.8452	11.7651	0.8939	11.1892	0.318	64.4%
WWTP_2_Pri	7.9945	8.0102	8.0075	0.013	7.9985	0.004	69.2%
WWTP_2_Sec	10.8453	11.7824	11.6955	0.8502	11.1581	0.3128	63.2%
WWTP_3_BS	13.2825	14.4922	14.4102	1.1277	13.4664	0.1839	83.7%
WWTP_3_Sec	15.3833	16.3796	16.2964	0.9131	15.569	0.1857	79.7%
WWTP_4_BS	19.4315	20.4002	20.2782	0.8467	19.6834	0.2519	70.2%
WWTP_4_BS	16.6315	17.5157	17.405	0.7735	16.8593	0.2278	70.5%
WWTP_4_Sec	19.7815	20.7461	20.6453	0.8638	20.041	0.2595	70.0%

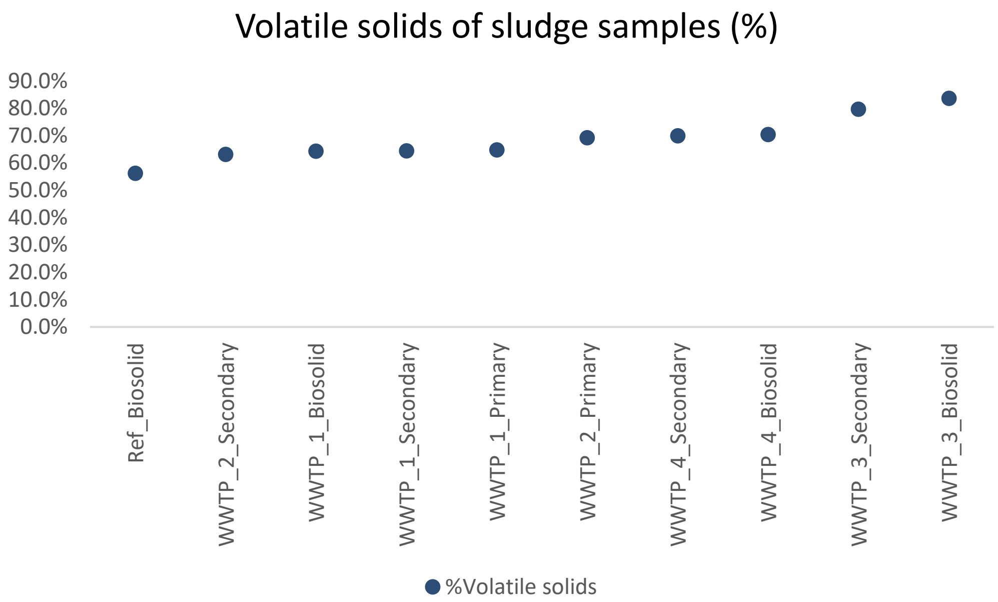


Figure 12-1 Volatile solids in sludge samples, sorted by quantity of volatile solids.

Statistical analysis for correlation between DOC and Vs was performed using SPSS. The results are presented in Table 12-3

Table 12-3 Correlation between leachable DOC and Vs measurement

Correlations

		DOC	Vs
DOC	Pearson Correlation	1	.138
	Sig. (2-tailed)		.703
	N	10	10
Vs	Pearson Correlation	.138	1
	Sig. (2-tailed)	.703	
	N	10	10

Appendix 4 Calculation of Limit of Detection for TOC analysis

The equipment used to analyse samples for TOC was found to have manufacturer stated detection limits, of < 1 $mg{\cdot}L^{-1}$ suitable for the needs of this study. However, due to the age of the instrument, and no previous work to verify detection limits, a short experiment was conducted to calculate instrument detection limit. This experiment was based upon previous experience working in method development and validation in ISO17025 accredited laboratories (ALS Environmental, Vancouver B.C., and Scottish Environment Protection Agency, Riccarton U.K.) as well as guidance from Armbruster and Pry (2008).

12.4.1 Background

Limits of detection (LOD) and limits of quantification (LOQ) are often used to define the lower level analytical capabilities of an instrument. Typically, LOD is defined as the lowest limit at which an analyte can be detected but not necessarily quantified, and the LOQ is the lowest limit at which an analyte can be reliably quantified (usually higher than the LOD). In industry, the terminology “LOD” is often used in place of LOQ, however having a safety factor applied to the true LOD (e.g. 2 or 3 times). Limit of Blank (LOB) is also used in calculating lower analytical capability of instrumentation. The LOB is defined as the highest signal that is expected to be returned for a sample containing no analyte, or a measure of the signal/noise.

The limit of detection (LOD) used in analytical laboratories typically utilise value based on the standard deviation of a set of blank values (SD, σ) multiplied by a safety factor of 2 or 3.

$$\text{e.g. LOD} = 3\sigma_{\text{blank}}$$

Alternative methods of calculating instrument limits are described by Armbruster and Pry (2008). Limit of the blank (e.g. the level at which a difference can be detected between a sample with analyte vs a sample with no analyte) using alternative methods can be carried out using the mean plus a 95% confidence limit e.g. limit of blank (LOB) (95% confidence) = μ_{blank} plus 1.645 (σ_{blank})

limit of detection (LOD) = LOB plus 1.645($\sigma_{low\ concentration\ sample}$)

12.4.2 Method

Repeated measures of a blank sample (distilled deionised water) and a low concentration sample (1 mg·L^{-1}) sample were performed in a single analytical run (analysis 140817). Twenty replicates of each was used in determination of LOB and LOD.

12.4.3 Results

A comparison of LOD calculation methods in the current study is presented in Table 12-4. The μ_{blank} was calculated to be 0.39 mg·L^{-1}, and σ_{blank} was calculated to be 0.066. The $\mu_{1\ mg.L-1}$was calculated to be 1.22 mg·L^{-1}, and the σ_{1ppm} was calculated to be 0.17.

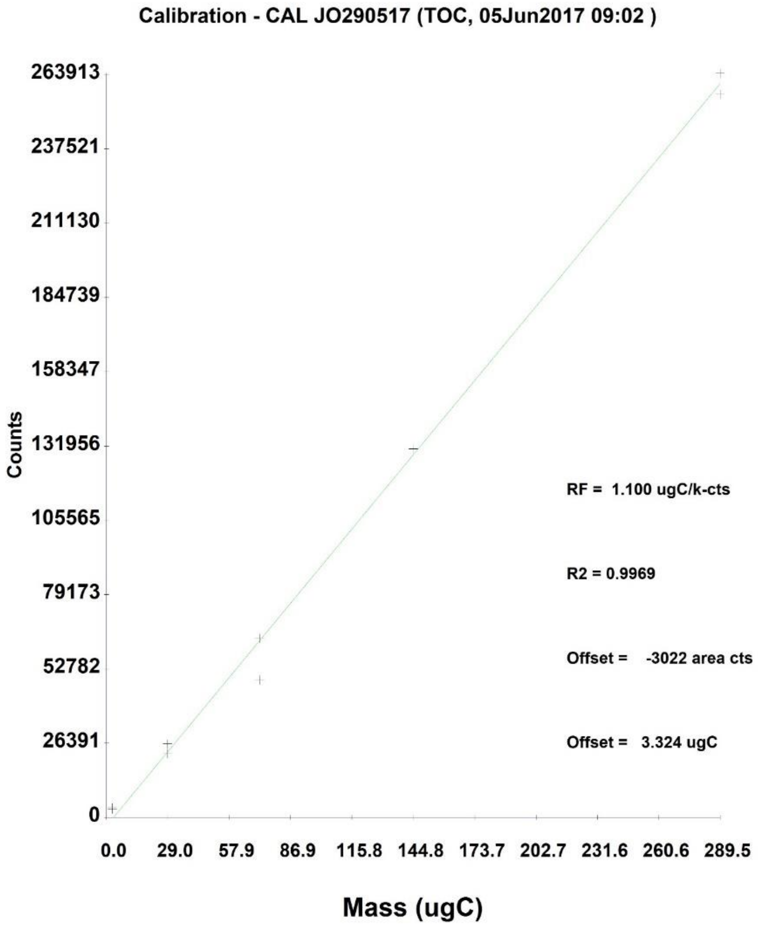
Table 12-4 Comparison of methodology of LOD calculations for TOC analysis

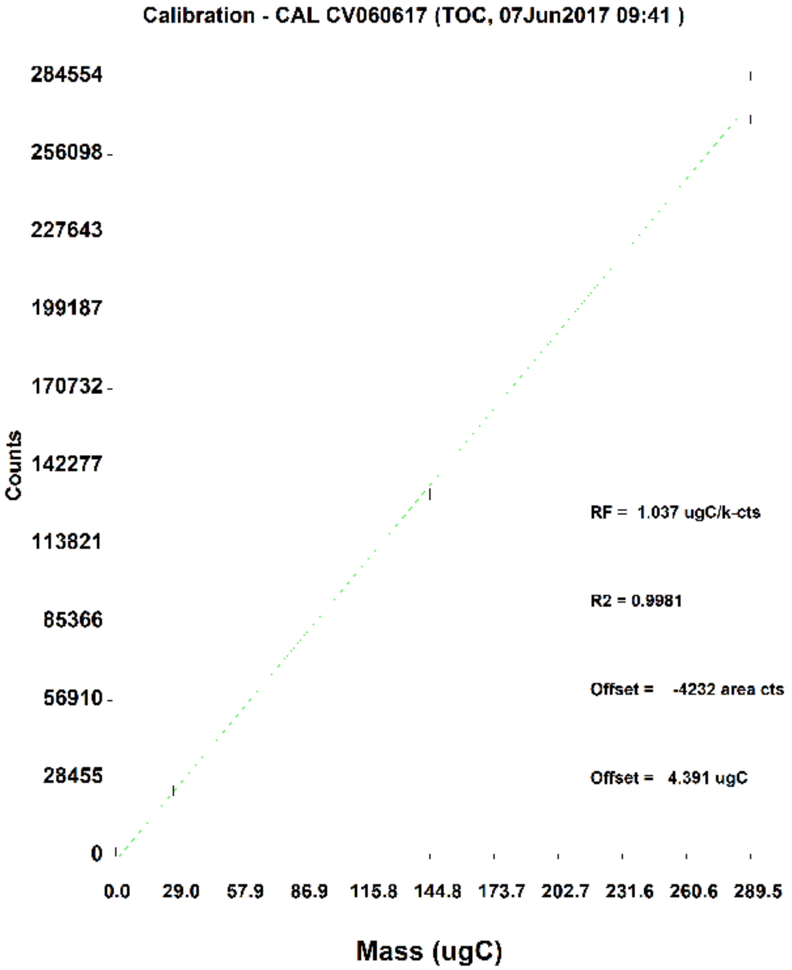
Method of calculation	**Result for this study based on n=20**
LOB (μ_{blank} + 1.645* σ_{blank})	0.49 mg·L^{-1}
LOD (2*σ_{blank})	0.77 mg·L^{-1}
LOD (3*σ_{blank})	1.16 mg·L^{-1}
LOD (LOB + 1.645* $\sigma_{1\ mg.L-1}$)	0.67 mg·L^{-1}

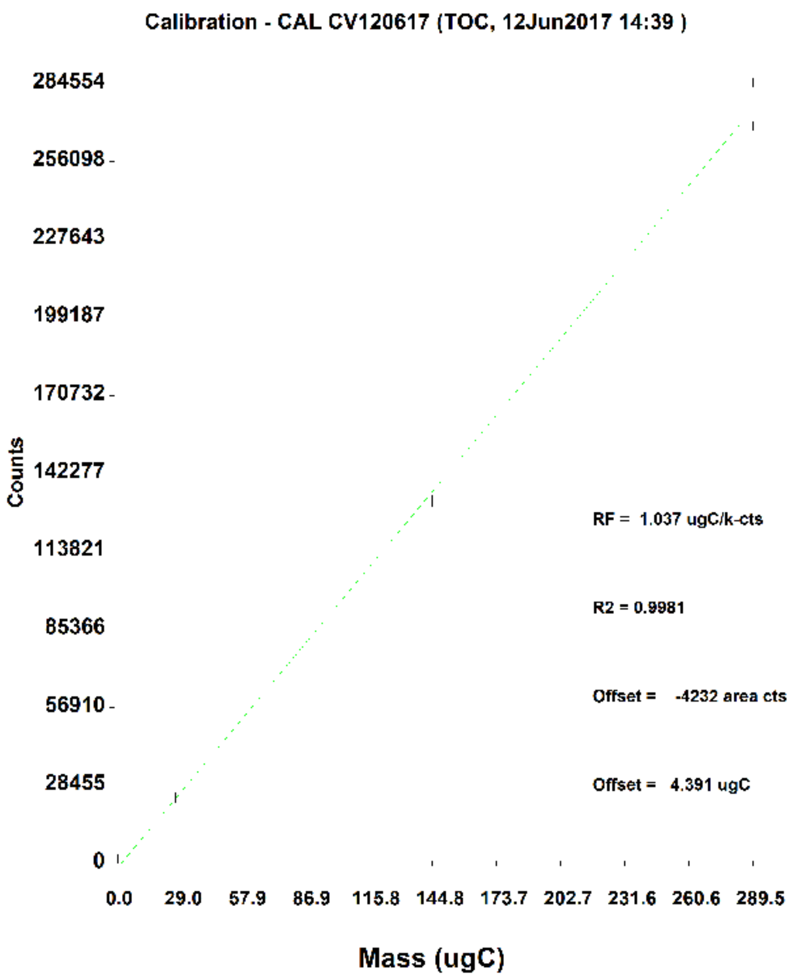
Based on the table above, it appears that a LOB of >0.5 ppm is achievable based on calculated results. The LOD calculation method provide an indication that LOD between 0.7 and 1.2 ppm is likely achievable. Prior to this analysis, several 5-point calibrations were carried out using a 1 to 100 mg·L^{-1} set of calibration standards. These calibrations were round to achieve an R^2 value in excess of 0.99, indicating a good linear relationship between 1 and 100 mg·L^{-1}. The mean value obtained in the validation study for a 1 mg·L^{-1} sample was calculated to be 1.22 suggesting a slightly positive bias for samples around 1ppm. In order to ensure the accuracy of samples in this study, a LOD of 1.2 is thus used, falling in line with the calculation using 3*$\sigma_{blank.}$ Thus, results falling below 1.2 mg·L^{-1} in this study, will be classified as < LOD for the purposes of the current study. Where a dilution factor has been used, the LOD is multiplied by the dilution factor where relevant, and samples falling below the

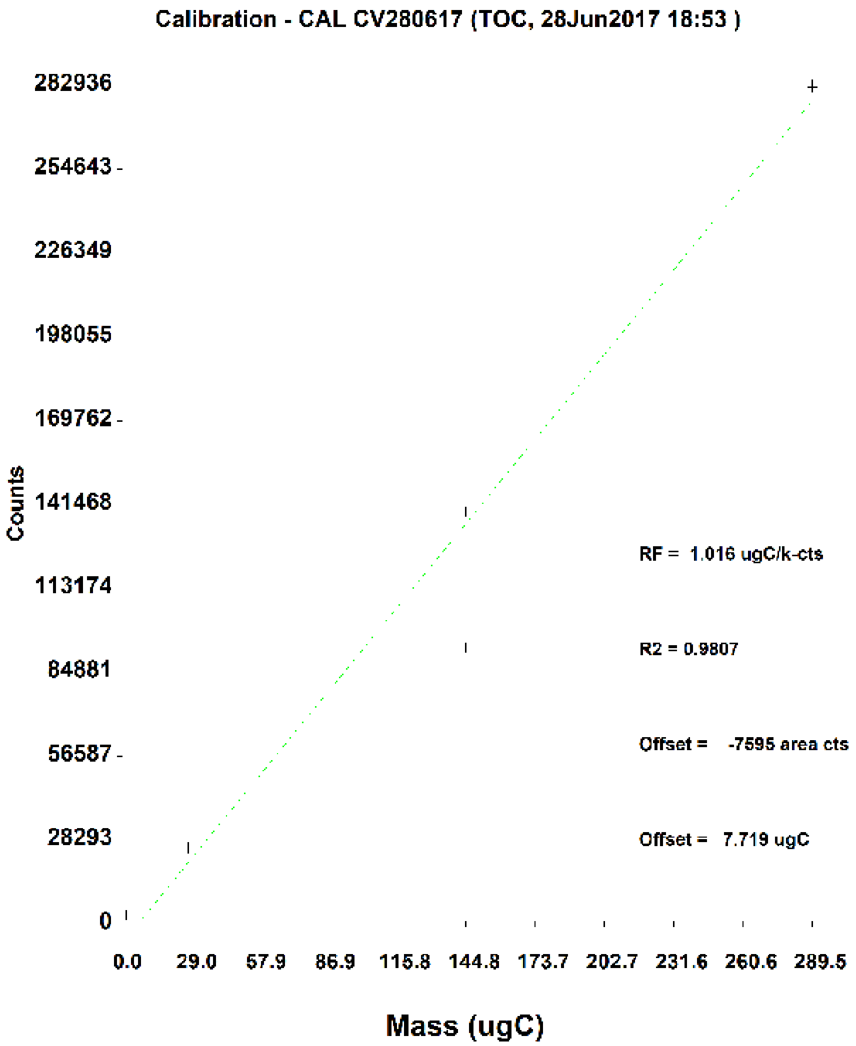
LOD, where a dilution has been used will be reanalysed using a lower dilution factor or neat sample where possible. Due to the low volume of sample available for some sampling points or replicates in this study, a repeat neat analysis for low samples was not possible for all samples.

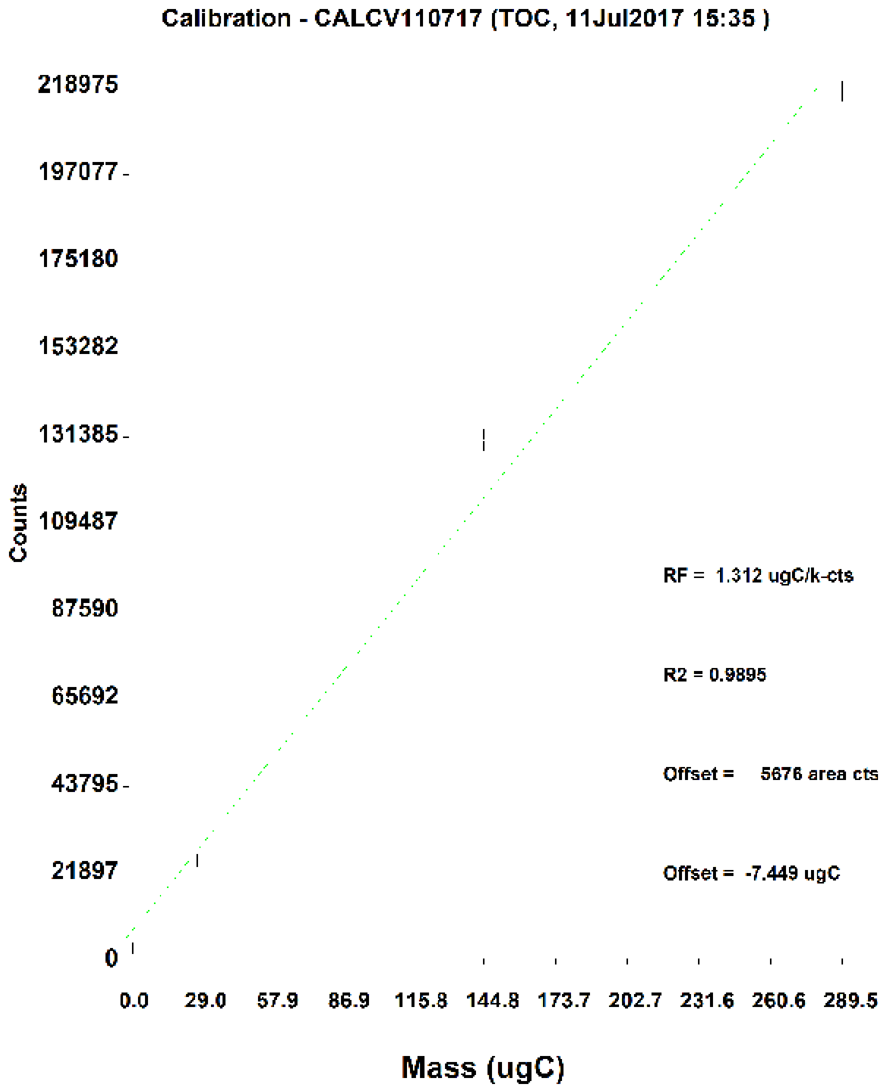
Appendix 5 Calibration curves for *OI Analytical* 1010 TOC analyser

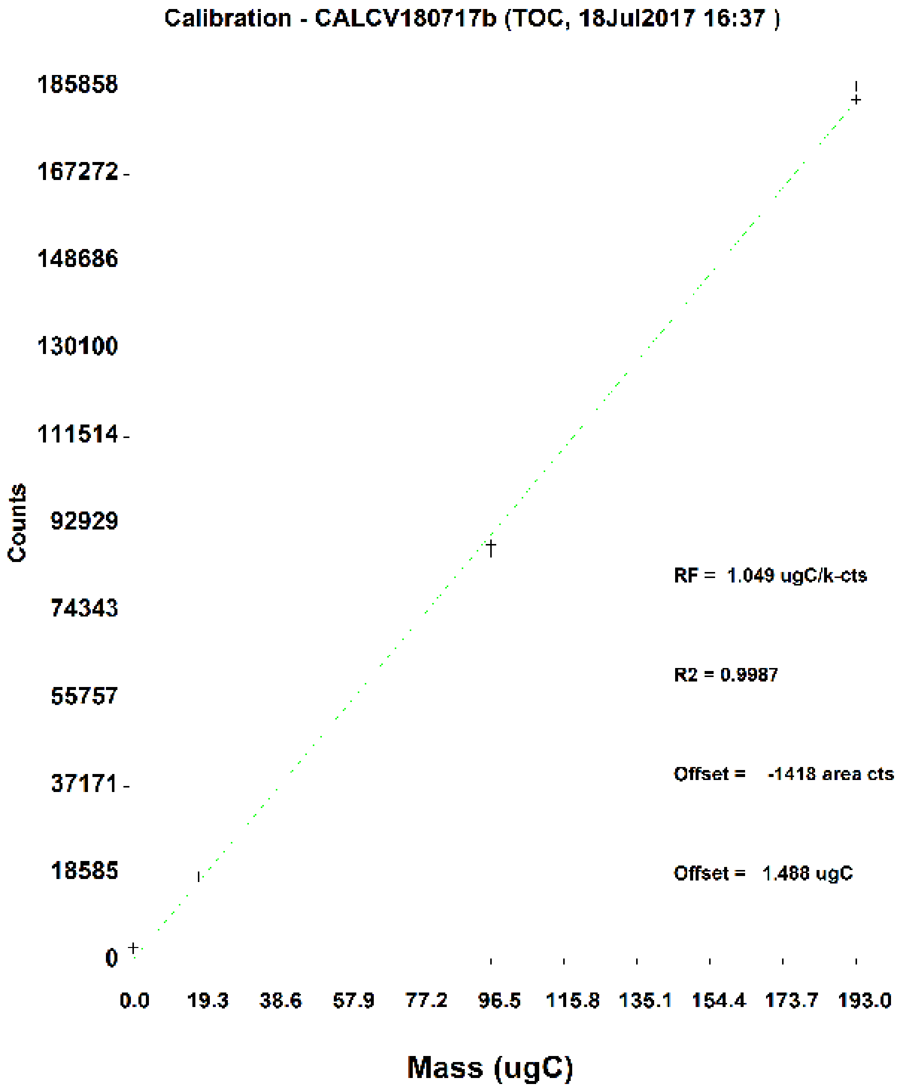





Calibration - CAL CV120617 (TOC, 12Jun2017 14:39)
284554
256098
227643
199187
170732
142277
113821
85366
56910
28455
0
Counts
0.0
29.0
57.9
86.9
115.8
144.8
173.7
202.7
231.6
260.6
289.5
Mass (ugC)
RF = 1.037 ugC/k-cts
R2 = 0.9981
Offset = -4232 area cts
Offset = 4.391 ugC


Calibration - CAL CV280617 (TOC, 28Jun2017 18:53)
282936
254643
226349
198055
169762
141468
113174
84881
56587
28293
0
Counts
0.0
29.0
57.9
86.9
115.8
144.8
173.7
202.7
231.6
260.6
289.5
Mass (ugC)
RF = 1.016 ugC/k-cts
R2 = 0.9807
Offset = -7595 area cts
Offset = 7.719 ugC


Calibration - CALCV110717 (TOC, 11Jul2017 15:35)
Counts
218975
197077
175180
153282
131385
109487
87590
65692
43795
21897
0
RF = 1.312 ugC/k-cts
R2 = 0.9895
Offset = 5676 area cts
Offset = -7.449 ugC
0.0
29.0
57.9
86.9
115.8
144.8
173.7
202.7
231.6
260.6
289.5
Mass (ugC)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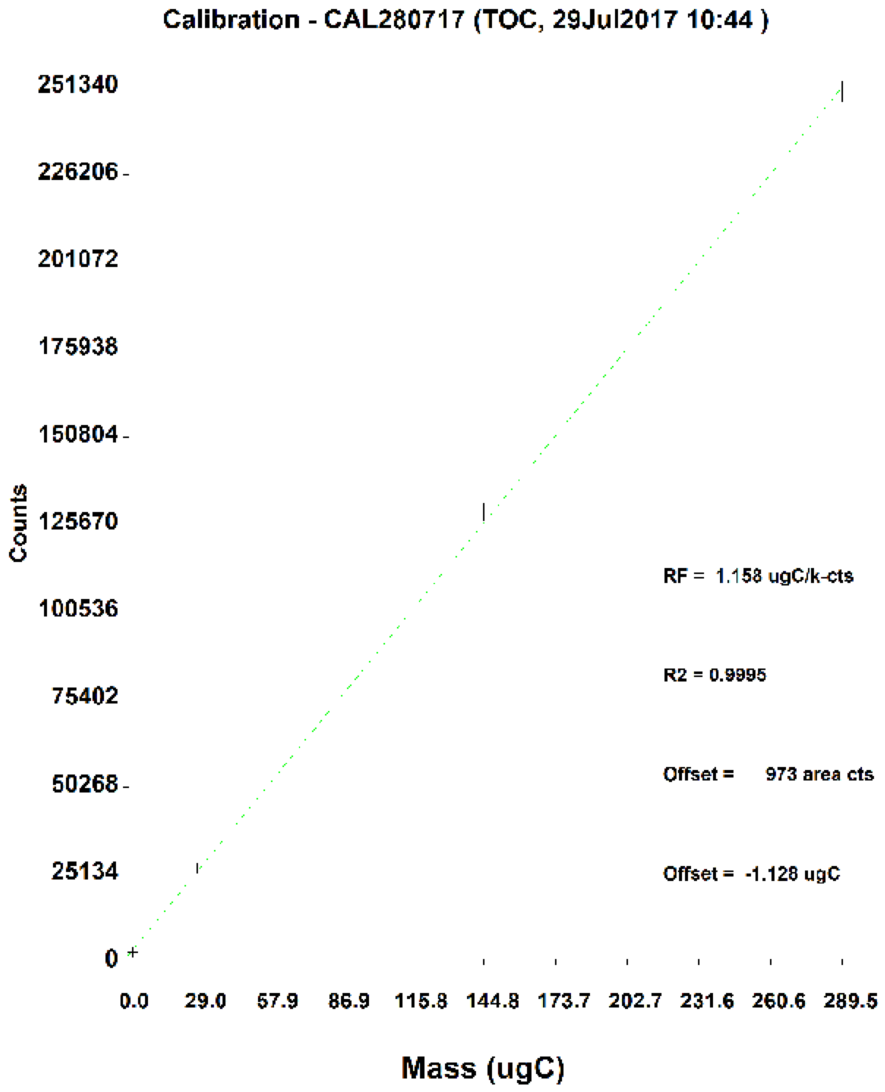
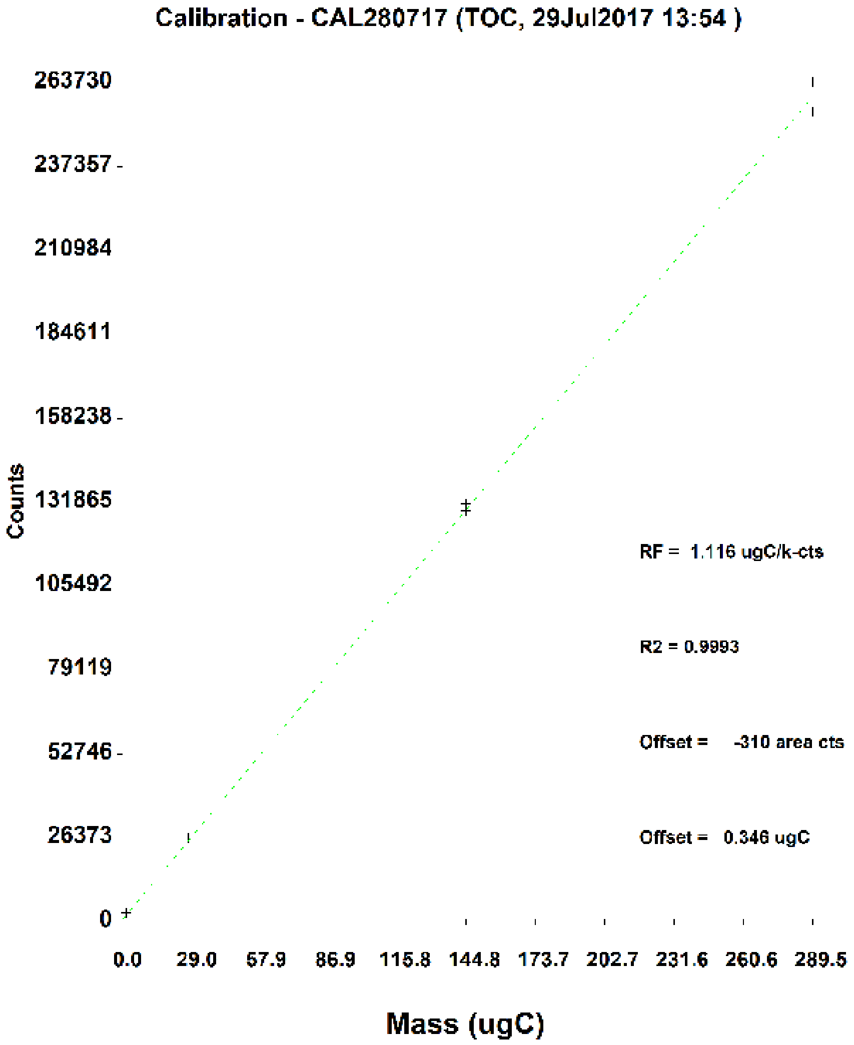
Counts
185858
167272
148686
130100
111514
92929
74343
55757
37171
18585
0
RF = 1.049 ugC/k-cts
R2 = 0.9987
Offset = -1418 area cts
Offset = 1.488 ugC
0.0
19.3
38.6
57.9
77.2
96.5
115.8
135.1
154.4
173.7
193.0
Mass (ugC)


Calibration - CAL280717 (TOC, 29Jul2017 10:44)
Counts
251340
226206
201072
175938
150804
125670
100536
75402
50268
25134
0
0.0
29.0
57.9
86.9
115.8
144.8
173.7
202.7
231.6
260.6
289.5
Mass (ugC)
RF = 1.158 ugC/k-cts
R2 = 0.9995
Offset = 973 area cts
Offset = -1.128 ugC


Calibration - CAL280717 (TOC, 29Jul2017 13:54)
Counts
263730
237357
210984
184611
158238
131865
105492
79119
52746
26373
0
0.0
29.0
57.9
86.9
115.8
144.8
173.7
202.7
231.6
260.6
289.5
Mass (ugC)
RF = 1.116 ugC/k-cts
R2 = 0.9993
Offset = -310 area cts
Offset = 0.346 ugC

Appendix 6 Effects of shaking time and pH on DDOC

12.6.1 Method

To assess the effect of shaking time and pH on leachable DOC, an experiment testing pH at three levels (4.2, 7 and 9) and shaking time at 4 levels (4, 8, 18 and 24 h) was carried out using the biosolid from WWTP_1, in the form of pellets, that have been pre-treatment by AD, dewatered and dried. This site and biosolid was selected over the other biosolids for several principle reasons. First the sample is gathered from a typical large WWTP, similar in configuration to many others present in the UK today. The processing of the pellets also ensures that the samples provide a relatively homogenous sub-sample of the typical biosolid by-product of these types of plants. In comparison, sludge from WWTP_2 and WWTP_4 are often collected and transported to a large WWTP for processing there. Also, this material is actively sold as a biosolid for application to land, so is known to be in use, as opposed to the solids from WWTP_3, which are disposed of in landfill. A Taguchi orthogonal array (SPSS) was used to assign subsamples of sludge randomly to various treatment conditions as shown in Table 12-5 with treatments A1 to D9 providing triplicate analysis at each combination of test conditions.

Table 12-5 Taguchi array for assignment of pH and shaking time treatments.

	A	**B**	**C**	**D**
	4 h	8 h	18 h	24h
1	pH 4.2	pH 4.2	pH 4.2	pH 4.2
2	pH 4.2	pH 7	pH 7	pH 7
3	pH 4.2	pH 9	pH 9	pH 9
4	pH 7	pH 4.2	pH 7	pH 9
5	pH 7	pH 7	pH 9	pH 4.2
6	pH 7	pH 9	pH 4.2	pH 7
7	pH 9	pH 4.2	pH 9	pH 7
8	pH 9	pH 7	pH 4.2	pH 9
9	pH 9	pH 9	pH 7	pH 4.2

Moisture content determination was carried out as described in Chapter 6 on a duplicate sample of WWTP_1 biosolids. The mean moisture content was calculated to be 7.44%, and this figure was used to calculate dry weight for all samples based on measured sample mass of approximately 1 g. Extraction fluid of 50.0 mL was used for all samples. Samples were extracted at a turning speed of 30 revolutions per minute (rpm). At the end of each allocated extraction time, samples were removed from the turning apparatus, centrifuged for 5 minutes at 3000 rpm, and filtered through 0.45 µm filter paper. Samples were analysed for DOC using the *OI Analytical* 1010 TOC analyser as described in Chapter 6. Final pH was measured at the end of each extraction procedure using a handheld pH meter (*Hach* Sension 3). Starting pH was recorded as the pH of the extraction fluid (4.2, 7.0 or 9.0).

12.6.2 Results

Sample extraction date, time, starting and final pH and desorbable DOC results are presented in Table 12-6.

Table 12-6 Sample extraction details, final pH and DOC results

Date	Sample	Start time	End time	Starting pH	Shaking time (h)	Dry weight (g)	final pH	DDOC (mg/l)	DDOC (mg/kg)
23/02/18	A1	08:15	12:15	4.2	4	0.9272	6.01	191.0	10297.1
23/02/18	A2	08:15	12:15	4.2	4	0.9347	6.65	342.5	18322.5
23/02/18	A3	08:15	12:15	4.2	4	0.9302	6.77	320.4	17222.0
23/02/18	B1	08:15	16:15	4.2	8	0.9256	6.62	440.0	23769.5
23/02/18	B2	08:15	16:15	7	8	0.9247	6.83	307.2	16612.2
23/02/18	B3	08:15	16:15	9	8	0.9252	7.01	372.8	20145.2
05/03/18	C1	15:40	09:40	4.2	18	0.9369	6.76	234.9	12534.2
05/03/18	C2	15:40	09:40	7	18	0.9341	6.82	322.4	17255.3
05/03/18	C3	15:40	09:40	9	18	0.9310	6.83	429.3	23052.3
26/02/18	D1	08:15	08:15+24	4.2	24	0.9330	6.67	401.4	21514.2
26/02/18	D2	08:15	08:15+24	7	24	0.9178	6.82	413.0	22497.1
26/02/18	D3	08:15	08:15+24	9	24	0.9175	6.89	432.4	23560.8
23/02/18	A4	08:15	12:15	7	4	0.9274	6.91	329.0	17734.8
23/02/18	A5	08:15	12:15	7	4	0.9274	7.11	308.2	16615.9
23/02/18	A6	08:15	12:15	7	4	0.9200	6.88	314.5	17090.4
07/03/18	B4	08:45	16:45	4.2	8	0.9495	6.66	380.7	20047.7
07/03/18	B5	08:45	16:45	7	8	0.9483	6.80	335.7	17698.8
07/03/18	B6	08:45	16:45	9	8	0.9247	6.84	380.1	20553.3
05/03/18	C4	15:40	09:40	7	18	0.9322	6.86	449.3	24098.7
05/03/18	C5	15:40	09:40	9	18	0.9231	6.85	426.2	23086.6
05/03/18	C6	15:40	09:40	4.2	18	0.9370	6.75	395.4	21101.5
26/02/18	D4	08:15	08:15+24	9	24	0.9230	6.90	439.5	23806.8
26/02/18	D5	08:15	08:15+24	4.2	24	0.9206	6.78	448.9	24383.9
26/02/18	D6	08:15	08:15+24	7	24	0.9291	6.84	428.8	23075.0
23/02/18	A7	12:15	16:15	9	4	0.9230	6.78	361.8	19601.5
23/02/18	A8	12:15	16:15	9	4	0.9383	6.91	342.4	18245.5
23/02/18	A9	12:15	16:15	9	4	0.9246	6.83	328.2	17747.3
07/03/18	B7	08:45	16:45	4.2	8	0.9417	6.67	400.3	21253.1
07/03/18	B8	08:45	16:45	7	8	0.9359	6.76	340.0	18164.6

Date	Sample	Start time	End time	Starting pH	Shaking time (h)	Dry weight (g)	final pH	DDOC (mg/l)	DDOC (mg/kg)
07/03/18	B9	08:45	16:45	9	8	0.9396	6.91	248.5	13220.8
05/03/18	C7	15:40	09:40	9	18	0.9214	6.93	360.8	19578.2
05/03/18	C8	15:40	09:40	4.2	18	0.9247	6.76	431.3	23320.1
05/03/18	C9	15:40	09:40	7	18	0.9401	6.72	349.6	18594.8
26/02/18	D7	08:15	08:15+24	7	24	0.9210	6.85	424.4	23038.9
26/02/18	D8	08:15	08:15+24	9	24	0.9225	6.86	442.5	23981.8
26/02/18	D9	08:15	08:15+24	4.2	24	0.9251	6.78	429.4	23207.8

Statistical software SPSS (*IBM* version 25, 2017) was used to analyse the results of this study. Descriptive statistics (mean and standard deviation) are presented in Table 12-7.

Table 12-7 Descriptive statistics for DOC at various pH and shaking time levels

Descriptive Statistics

Dependent Variable: DOC

ShakeTime	StartpH	Mean	SD	N
4 hours	pH 4.2	15280.5080	4350.73268	3
	pH7	17147.0230	561.59524	3
	pH 9.4	18531.4289	959.60516	3
	Total	16986.3200	2652.80883	9
8 hours	pH 4.2	21690.0872	1899.02978	3
	pH7	17491.8564	796.58803	3
	pH 9.4	17973.1029	4120.67767	3
	Total	19051.6822	3043.73156	9
18 hours	pH 4.2	18985.2551	5695.83287	3
	pH7	19982.9325	3626.75880	3
	pH 9.4	21905.6902	2015.77998	3
	Total	20291.2926	3750.66359	9
24 hours	pH 4.2	23035.3084	1442.59775	3
	pH7	22870.3361	323.73980	3
	pH 9.4	23783.1147	211.50090	3
	Total	23229.5864	857.38348	9
Total	pH 4.2	19747.7897	4466.77300	12
	pH7	19373.0370	2887.39286	12
	pH 9.4	20548.3342	3205.61561	12
	Total	19889.7203	3516.76112	36

The test of between subjects effects identified that shaking time had a significant effect on DDOC (p = 0.000), starting pH did not have a significant effect (p = 0.580) Figure 12-2 shows how mean DOC varied by pH and shaking time.

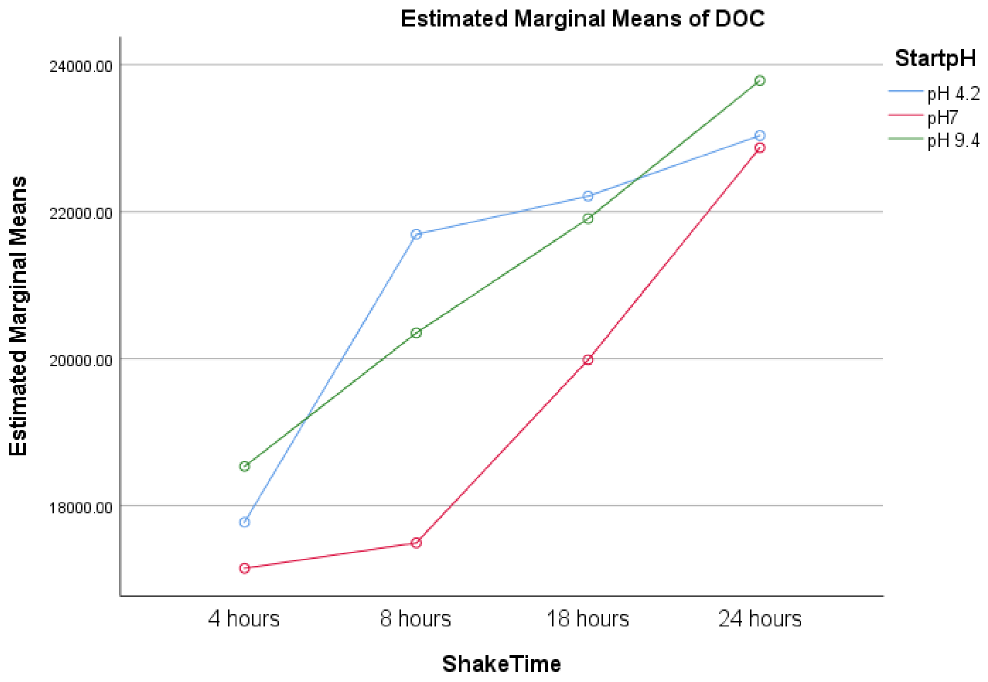


Figure 12-2 Estimated marginal means of DOC at various shaking time and starting pH combinations

A comparison of effect of starting pH on final pH was performed. Comparison of significant of difference (*significance at p* < *0.05*) between leachates at 4.2, 7 and 9 for all extract times is shown in Table 12-8. No significant difference is found between leachates with a starting pH of 7.0 and 9.0, however a significant difference is found between pH 4.2 and both pH 7.0 and pH 9.0 although the difference is small (-0.19 and -0.22 pH units respectively). The graphical presentation in Figure 12-3 depicts the mean and standard deviations of final pH for each starting pH level.

Table 12-8 Multiple comparison of DDOC across pH levels

Multiple Comparisons						
Dependent Variable: pH_Final						
Tukey HSD						
(I) Leachate_pH	(J) Leachate_pH	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
4.2	7	-.19333*	.05662	**.005**	-.3323	-.0544
	9	-.22167*	.05662	**.001**	-.3606	-.0827
7	4.2	.19333*	.05662	.005	.0544	.3323
	9	-.02833	.05662	.872	-.1673	.1106
9	4.2	.22167*	.05662	.001	.0827	.3606
	7	.02833	.05662	.872	-.1106	.1673

*. The mean difference is significant at the 0.05 level.

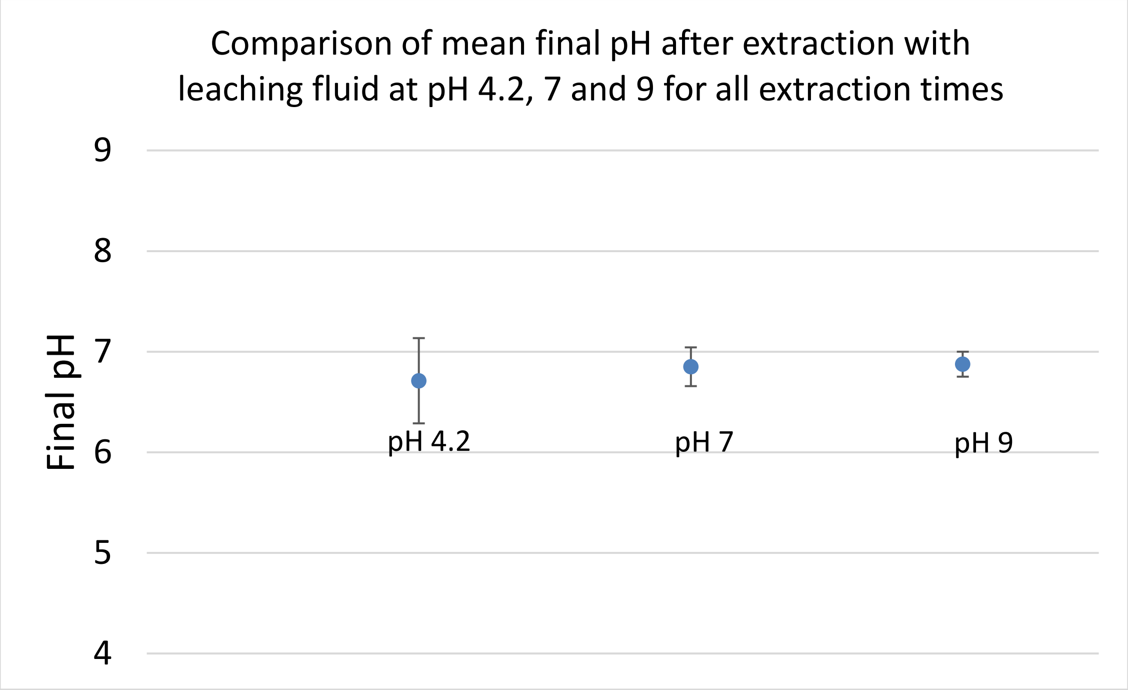


Figure 12-3 Comparison of final pH of all leachates. Error bars depict 2 x SD.

The data collected suggests that the pH of the leachate is relatively stable within 4 hours, and does not change significantly after this time, regardless of starting pH. The starting pH may therefore be less important than extraction time. A comparison of starting pH and final pH for all samples at all extraction times is shown in **Error! Reference source not found.**Figure 12-4. The data indicates a similar maximum DDOC achieved at each pH level, although higher variability in pH 9 samples was observed.

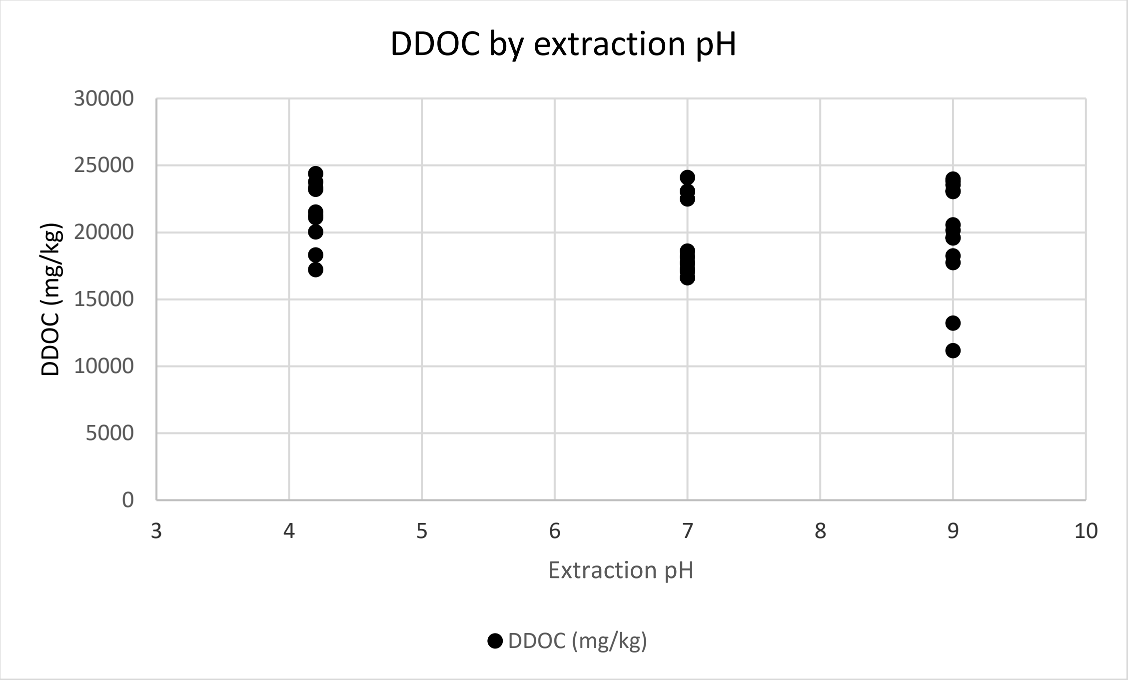


Figure 12-4 Comparison of effect of pH on DDOC

A comparison of the effect of shaking time on DDOC is presented in Figure 12-5 The results show that shaking time is lowest at 4 h and highest at 24 h, with greater variability shown in results at the middle times of 8 and 18 h.

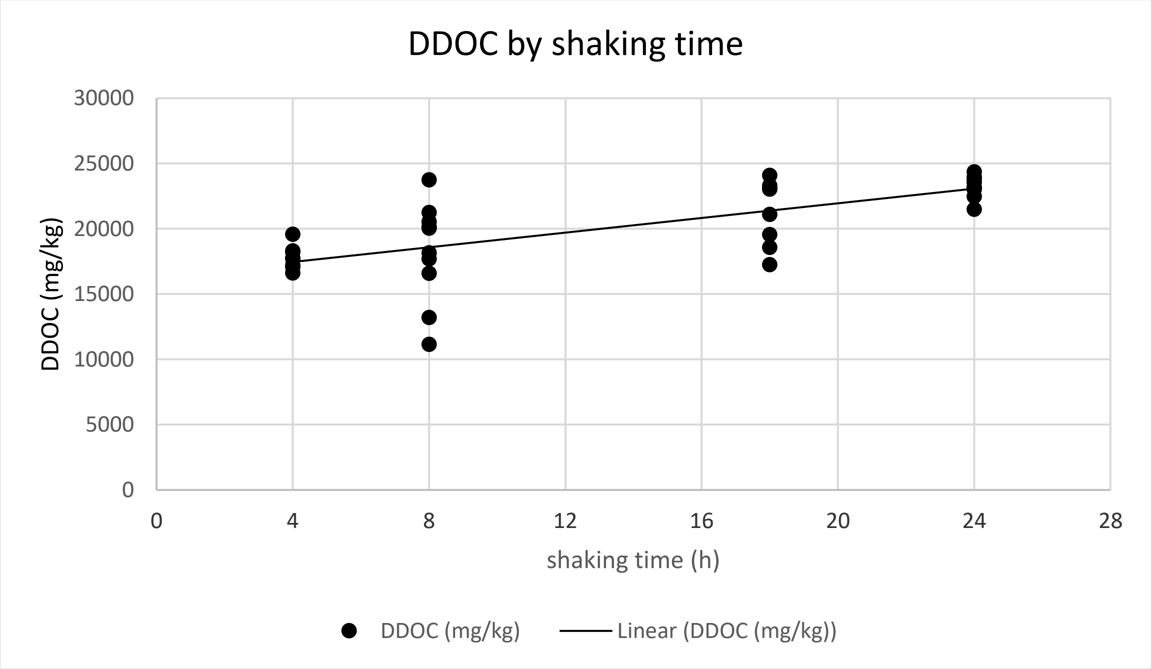


Figure 12-5 Comparison of the effect of shaking time on DDOC

12.6.3 Discussion and Conclusions

This test was carried out on pellets, where addition of chemical coagulations (polymers) and lime stabilisation occurs, as is common for many sludges, and therefore represents a typical sludge material. The data collected suggests that the pH of the leachate is relatively stable within 4 hours, and does not change significantly after this time, regardless of starting pH. The starting pH may therefore be less important than extraction time. The study of the effect of pH and shaking time undertaken in this study indicated that shaking time had a significant effect on DDOC, with a shaking time between 18 and 24 hours representing the maximum leachate DDOC concentration. The buffering capacity of the samples resulted in increasing pH over the extraction period.

Appendix 7 Results of DOC desorption experiments

Table 12-9 Leachate final pH from DDOC experiment

Sample Name	Sample type	Replicate	Final pH	Sample Name	Sample type	Replicate	Final pH
Reference	(biosolid)	1	6.56				
Reference	(biosolid)	2	6.61	**WWTP_2**	primary settled sludge	1	6.23
Reference	(biosolid)	3	6.59	**WWTP_2**	primary settled sludge	2	6.19
WWTP_1	primary settled sludge	1	7.28	**WWTP_2**	primary settled sludge	3	6.11
WWTP_1	primary settled sludge	1	7.06	**WWTP_2**	primary settled sludge	4	6.17
WWTP_1	primary settled sludge	2	7.43	**WWTP_2**	secondary settled sludge	1	6.71
WWTP_1	primary settled sludge	2	7.26	**WWTP_2**	secondary settled sludge	2	6.74
WWTP_1	primary settled sludge	3	7.25	**WWTP_2**	secondary settled sludge	3	6.65
WWTP_1	primary settled sludge	3	7.18	**WWTP_3**	secondary settled sludge	1	6.27
WWTP_1	primary settled sludge	4	7.14	**WWTP_3**	secondary settled sludge	2	6.29
WWTP_1	primary settled sludge	4	6.92	**WWTP_3**	secondary settled sludge	3	6.28
WWTP_1	secondary settled sludge	1	6.75	**WWTP_3**	sludge cake (biosolid)	1	6.86
WWTP_1	secondary settled sludge	2	6.82	**WWTP_3**	sludge cake (biosolid)	2	6.84
WWTP_1	secondary settled sludge	2	6.66	**WWTP_3**	sludge cake (biosolid)	3	6.88
WWTP_1	secondary settled sludge	3	6.89	**WWTP_4**	secondary settled sludge	1	6.36
WWTP_1	secondary settled sludge	3	6.75	**WWTP_4**	secondary settled sludge	2	6.36
WWTP_1	secondary settled sludge	4	6.81	**WWTP_4**	secondary settled sludge	3	6.36
WWTP_1	sludge pellet (biosolid)	1	6.89				
WWTP_1	sludge pellet (biosolid)	1	6.75				
WWTP_1	sludge pellet (biosolid)	2	6.8				
WWTP_1	sludge pellet (biosolid)	3	6.78				
WWTP_1	sludge pellet (biosolid)	3	6.78				
						Min	6.11
						Max	7.43

Table 12-10 Results of DDOC experiment

Site	Leachate Batch	Sample location and type	Date collected	Label ID	Leachate Date	Date analysed	Mass of sample (g)	Moisture %	dw (g)	Leachate vol	DOC (mg.L^{-1})	DDOC (mg.kg^{-1} dw)	Average	SD
1	1	Biosolids (Apr)	12/4/17	WWTP_1_BS_1204_1	5/6/17	6/6/17	2.4960	5.17%	2.3670	50.0	1146.09	24,210.3	22,542.4	2,358.8
1	1	Biosolids (Apr)	12/4/17	WWTP_1_BS_1204_3	5/6/17	6/6/17	2.5070	5.17%	2.3774	49.0	1012.79	20,874.5		
1	1	Biosolids (Old)	pre 2017	WWTP_1_BS_OLD_1	5/6/17	6/6/17	2.4980	6.56%	2.3341	50.0	1279.72	27,413.2	27,862.4	1,740.6
1	1	Biosolids (Old)	pre 2017	WWTP_1_BS_OLD_2	5/6/17	6/6/17	2.5020	6.56%	2.3379	50.0	1392.60	29,783.5		
1	1	Biosolids (Old)	pre 2017	WWTP_1_BS_OLD_3	5/6/17	6/6/17	2.5040	6.56%	2.3397	50.0	1234.93	26,390.4		
1	2	Primary (Apr)	12/4/17	WWTP_1_Pri_1204_1	7/6/17	13/6/17	2.5015	11.66%	2.2098	48.5	1020.23	22,391.3	21,691.8	746.0
1	2	Primary (Apr)	12/4/17	WWTP_1_Pri_1204_2	7/6/17	13/6/17	2.5029	11.66%	2.2111	48.5	985.47	21,616.3		
1	2	Primary (Apr)	12/4/17	WWTP_1_Pri_1204_3	7/6/17	13/6/17	2.4969	11.66%	2.2058	48.0	1014.63	22,079.6		
1	7	Primary (Apr)	12/4/17	WWTP_1_Pri_1204_4	4/7/17	11/7/17	2.5023	11.66%	2.2105	48.0	952.36	20,679.8		
1	2	Secondary (Apr)	12/4/17	WWTP_1_Sec_1204_1	7/6/17	13/6/17	2.5003	13.10%	2.1728	49.0	981.00	22,123.5	22,864.3	1,010.7
1	2	Secondary (Apr)	12/4/17	WWTP_1_Sec_1204_2	7/6/17	13/6/17	2.4993	13.10%	2.1719	49.0	1078.91	24,341.2		
1	2	Secondary (Apr)	12/4/17	WWTP_1_Sec_1204_3	7/6/17	13/6/17	2.502	13.10%	2.1742	49.0	1006.13	22,674.8		
1	7	Secondary (Apr)	12/4/17	WWTP_1_Sec_1204_4	4/7/17	11/7/17	2.5032	13.10%	2.1753	48.0	1011.40	22,317.6		
1	3	Primary (May)	8/5/17	WWTP_1_Pri_0805_1	13/6/17	15/6/17	2.5021	10.12%	2.2489	48.0	1225.64	26,159.8	21,985.5	3,128.5
1	3	Primary (May)	8/5/17	WWTP_1_Pri_0805_2	13/6/17	15/6/17	2.5010	10.12%	2.2479	48.0	1051.88	22,461.1		
1	3	Primary (May)	8/5/17	WWTP_1_Pri_0805_3	13/6/17	15/6/17	2.5091	10.12%	2.2552	48.0	955.22	20,331.3		
1	7	Primary (May)	8/5/17	WWTP_1_Pri_0805_4	4/7/17	11/7/17	2.4982	10.12%	2.2454	48.0	888.32	18,989.7		
1	3	Secondary (May)	8/5/17	WWTP_1_Sec_0805_1	13/6/17	15/6/17	2.4983	10.75%	2.2297	48.0	1625.37	34,989.6	33,852.9	1,574.2
1	3	Secondary (May)	8/5/17	WWTP_1_Sec_0805_2	13/6/17	15/6/17	2.4980	10.75%	2.2295	48.0	1603.03	34,513.0		
1	3	Secondary (May)	8/5/17	WWTP_1_Sec_0805_3	13/6/17	15/6/17	2.5069	10.75%	2.2374	48.0	1494.22	32,056.0		
2	4	Primary	5/4/17	WWTP_2_Pri_0504_1	15/6/17	22/6/17	0.4935	7.50%	0.4565	48.0	232.84	24,483.4	26,536.8	1,519.2
2	4	Primary	5/4/17	WWTP_2_Pri_0504_2	15/6/17	22/6/17	0.4999	7.50%	0.4624	48.0	255.29	26,499.8		

Site	Leachate Batch	Sample location and type	Date collected	Label ID	Leachate Date	Date analysed	Mass of sample (g)	Moisture %	dw (g)	Leachate vol	DOC (mg.L^{-1})	DDOC (mg.kg^{-1} dw)	Average	SD
2	4	Primary	5/4/17	WWTP_2_Pri_0504_3	15/6/17	22/6/17	0.5075	7.50%	0.4694	48.0	264.76	27,071.5		
2	7	Primary	5/4/17	WWTP_2_Pri_0504_4	4/7/17	11/7/17	0.4962	7.50%	0.4590	48.0	268.63	28,092.4		
2	4	Secondary	5/4/17	WWTP_2_Sec_0504_1	15/6/17	22/6/17	0.9999	12.10%	0.8789	48.0	423.37	23,121.3	23,054.3	454.0
2	4	Secondary	5/4/17	WWTP_2_Sec_0504_2	15/6/17	22/6/17	1.0079	12.10%	0.8859	48.0	433.21	23,471.0		
2	4	Secondary	5/4/17	WWTP_2_Sec_0504_3	15/6/17	22/6/17	0.9958	12.10%	0.8753	48.0	411.59	22,570.5		
3	5	Secondary	12/4/17	WWTP_3_Sec_1204_1	22/6/17	28/6/17	0.9982	13.21%	0.8663	48.0	232.96	12,907.1	11,759.5	994.2
3	5	Secondary	12/4/17	WWTP_3_Sec_1204_2	22/6/17	28/6/17	1.0055	13.21%	0.8727	48.0	202.82	11,155.8		
3	5	Secondary	12/4/17	WWTP_3_Sec_1204_3	22/6/17	28/6/17	1.0020	13.21%	0.8696	48.0	203.20	11,215.7		
3	5	Biosolid	12/4/17	WWTP_3_BS_1204_1	22/6/17	28/6/17	1.0230	13.58%	0.8841	48.0	560.28	30,419.9	29,983.2	1,158.9
3	5	Biosolid	12/4/17	WWTP_3_BS_1204_2	22/6/17	28/6/17	1.0240	13.58%	0.8849	48.0	568.95	30,860.3		
3	5	Biosolid	12/4/17	WWTP_3_BS_1204_3	22/6/17	28/6/17	1.0287	13.58%	0.8890	48.0	530.98	28,669.4		
4	6	Secondary	16/5/17	WWTP_4_Sec_1605_1	27/6/17	28/6/17	1.0055	12.54%	0.8794	48.0	392.93	21,447.1	20,275.3	1,089.4
4	6	Secondary	16/5/17	WWTP_4_Sec_1605_2	27/6/17	28/6/17	1.0060	12.54%	0.8798	48.0	353.65	19,293.4		
4	6	Secondary	16/5/17	WWTP_4_Sec_1605_3	27/6/17	28/6/17	0.9950	12.54%	0.8702	48.0	364.14	20,085.3		
4	8	Biosolid	27/2/18	WWTP_4_BS_1	27/2/18	28/2/18	0.9967	14.36%	0.8536	50.0	413.30	24,210.0	26,154.8	1,407.5
4	8	Biosolid	27/2/18	WWTP_4_BS_2	27/2/18	28/2/18	0.9846	14.36%	0.8432	50.0	442.89	26,262.3		
4	8	Biosolid	27/2/18	WWTP_4_BS_3	27/2/18	28/2/18	1.021	14.36%	0.8744	50.0	481.85	27,554.0		
4	8	Biosolid	27/2/18	WWTP_4_BS_4	27/2/18	28/2/18	0.9878	14.36%	0.8459	50.0	449.93	26,593.0		
REF	7	Biosolid	1/7/17	Ref_1	4/7/17	11/7/17	2.5013	13.46%	2.1646	48.0	635.19	14,085.1	14,421.9	322.6
REF	7	Biosolid	1/7/17	Ref_2	4/7/17	11/7/17	2.4991	13.46%	2.1627	48.0	651.19	14,452.6		
REF	7	Biosolid	1/7/17	Ref_3	4/7/17	11/7/17	2.5000	13.46%	2.1635	48.0	663.84	14,728.1		

Table 12-11 Summary of leachate blanks for DDOC experiments

Date	Blank concentration (mg·L^{-1}) (all 20x dilution as per samples)	Reported value (mg·L^{-1})
120617	-1.732	<20
130617	-8.797	<20
160617	2.175	<20
220617	4.476 3.793	<20 <20
280617	2.801 5.283	<20 <20

Table 12-12 Summary of spikes for DDOC experiments

Site	Batch	Sample type	Date collected	Label ID	Extract Date	Date analysed	Mass (g)	Moisture %	Dry weight (g)	vol (50 mL)	DOC (mg/L)	DDOC (mg/kg dw)
1	1	Biosolids (Apr)	12/4/17	WWTP_1_BS_1204_SPK	5/6/17	6/6/17	2.4950	5.17%	2.3660	46	1038.299	20,186.64
1	1	Biosolids (Old)	pre 2017	WWTP_1_BS_OLD_SPK	5/6/17	6/6/17	2.5020	6.56%	2.3379	47.5	1339.994	27,225.53
1	2	Primary (Apr)	12/4/17	WWTP_1_Pri_1204_SPK	7/6/17	13/6/17	2.499	11.66%	2.2076	46.5	963.037	20,284.87
1	2	Secondary (Apr)	12/4/17	WWTP_1_Sec_1204_SPK	7/6/17	13/6/17	2.4945	13.10%	2.1677	46.5	845.585	18,138.73
1	3	Primary (May)	8/5/17	WWTP_1_Pri_0805_SPK	13/6/17	15/6/17	2.4989	10.12%	2.2460	45.5	1192.698	24,161.84
1	3	Secondary (May)	8/5/17	WWTP_1_Sec_0805_SPK	13/6/17	15/6/17	1.6306	10.75%	1.4553	45.5	1199.309	37,496.16
2	4	Primary	5/4/17	WWTP_2_Pri_0504_SPK	15/6/17	22/6/17	0.5000	7.50%	0.4625	45.5	196.232	19,304.99
2	4	Secondary	5/4/17	WWTP_2_Sec_0504_SPK	15/6/17	22/6/17	0.9955	12.10%	0.8750	45.5	425.982	22,149.94
3	5	Secondary	12/4/17	WWTP_3_Sec_1204_SPK	22/6/17	28/6/17	0.9954	13.21%	0.8639	45.5	191.061	10,062.74
3	5	Biosolid (cake)	12/4/17	WWTP_3_BS_1204_SPK	22/6/17	28/6/17	1.0239	13.58%	0.8849	45.5	659.034	33,888.12
4	6	Secondary	16/5/17	WWTP_4_Sec_1605_SPK	27/6/17	28/6/17	0.9940	12.54%	0.8694	45.5	355.456	18,603.79
REF	7	Biosolid	1/7/17	Ref_SPK	4/7/17	11/7/17	2.4998	13.46%	2.1633	45.5	704.699	14,821.53

Appendix 8 Results of PDDOC experiments

Table 12-13 Results of PDDOC experiment

Label ID	D0 (mg/kg)	D1 (mg/kg)	D2 (mg/kg)	D7 (mg/kg)	D16 (mg/kg)	D28 (mg/kg)	AD-10 d (mg/kg)	AD-10 d blank adjusted (mg/kg)
WWTP_1_BS_1204_1	24210.3	29378.2	20892.0	13722.7	10951.0	7664.5		
WWTP_1_BS_1204_2	*	25557.7	21381.7	14238.1	11828.5	8731.3		
WWTP_1_BS_1204_3	20874.5	26919.8	18547.1	12332.5	10018.5	6586.4		
WWTP_1_BS_OLD_1	27413.2	34546.3	23284.5	15391.4	12625.5	8075.8	8467.8	8193.4
WWTP_1_BS_OLD_2	29783.5	24985.4	18164.2	13296.6	11049.9	6895.4	6859.9	6585.9
WWTP_1_BS_OLD_3	26390.4	29836.9	19233.2	14060.3	12057.2	7882.6	8262.1	7988.4
WWTP_1_Pri_1204_4	20679.8	20972.4	13152.8	9224.0	7243.5	5725.3		
WWTP_1_Pri_1204_2	21616.3	20591.6	12465.5	8734.0	6358.2	4811.5		
WWTP_1_Pri_1204_3	22079.6	21756.8	12778.6	8610.9	7455.8	5657.3		
WWTP_1_Sec_1204_1	22123.5	22007.0	10358.7	7396.8	6181.2	5580.2		
WWTP_1_Sec_1204_4	22317.6	24235.0	11920.3	7226.1	6251.6	3749.9		
WWTP_1_Sec_1204_3	22674.8	20810.9	12531.8	7864.7	6189.1	4600.9		
WWTP_1_Pri_0805_1	26159.8	19263.7	12507.3	9673.8	7290.0	4791.4	5656.1	5382.7
WWTP_1_Pri_0805_2	22461.1	22358.5	11708.3	9399.4	6872.8	4764.7	6027.0	5753.4
WWTP_1_Pri_0805_4	18989.7	20058.2	11539.1	9091.5	6496.8	4767.4	5341.6	5067.8
WWTP_1_Sec_0805_1	34989.6	31155.3	14316.7	8814.6	7300.2	4779.0	4894.8	4619.0
WWTP_1_Sec_0805_2	34513.0	37257.1	16321.0	9285.0	7269.5	4775.9	5132.2	4856.4
WWTP_1_Sec_0805_3	32056.0	31589.3	15676.3	8794.6	6496.6	5362.5	5821.9	5547.1
WWTP_2_Pri_0504_4	28092.4	11574.0	9147.8	7551.0	10818.7	5502.1		
WWTP_2_Pri_0504_2	26499.8	16177.7	9011.6	7883.1	12726.1	6343.0		
WWTP_2_Pri_0504_3	27071.5	11489.0	9404.0	7611.7	10940.7	7303.9		
WWTP_2_Sec_0504_1	23121.3	16262.2	12419.9	9177.7	8816.9	6060.1		
WWTP_2_Sec_0504_2	23471.0	19666.3	14321.3	8220.4	6871.9	5792.6		
WWTP_2_Sec_0504_3	22570.5	19406.2	11237.3	8162.1	8579.1	6252.7		
WWTP_3_Sec_1204_1	12907.1	11462.3	9095.2	5424.1	6992.4	5015.7	6571.7	5862.0

WWTP_3_Sec_1204_2	11155.8	13406.1	8132.4	5667.9	6874.9	5108.7	5228.4	4523.8
WWTP_3_Sec_1204_3	11215.7	14135.1	8794.5	5905.0	5147.4	4497.1	4935.4	4228.3
WWTP_3_BS_1204_1	30419.9	20693.8	14642.0	11193.2	11312.1	6494.2	9496.5	8801.0
WWTP_3_BS_1204_2	30860.3	22797.4	16367.4	10814.3	10479.4	6232.0	9455.6	8760.8
WWTP_3_BS_1204_3	28669.4	19310.3	15622.7	10409.9	12031.0	6520.8	9385.4	8693.8
WWTP_4_Sec_1605_1	21447.1	11387.4	7691.9	5168.9	6773.0	5037.6	0.0	0.0
WWTP_4_Sec_1605_2	19293.4	10871.0	7931.4	4887.7	6146.5	4026.2	0.0	0.0
WWTP_4_Sec_1605_3	20085.3	10579.8	7307.1	4926.5	7050.1	4114.8	0.0	0.0
Ref_1	14085.1	11374.2	9084.7	6924.1	7295.0	4875.8	6357.9	6073.9
Ref_2	14452.6	12210.3	9403.5	7084.0	7077.9	5151.3	5905.0	5620.7
Ref_3	14728.1	12497.5	9667.5	0.0	6408.5	5397.2	5391.0	5106.7

*** D0 sample analysis failure**

Table 12-14 Quality Control results for biodegradation batch blanks and spikes (results in mg·L^{-1})

Sample ID	D0	D1	D2	D7	D16	D28	D38 (AD)
Batch 1 for all WWTP_1 samples (Pri, Sec and BS) biodegradation experiments							
Batch 1 Blank1	4.98	3.44	2.86	3.02	8.52	4.94	22.86
Batch 1 Blank2	3.16	2.85	2.05	1.95	2.67	4.92	
Batch 1 Spike	112.97	118.45	87.77	69.02	57.25	40.94	
Batch 2 for all WWTP_ 2, 3, 4 and REF (Pri, Sec or BS) biodegradation experiments							
Batch 2 Blank1	4.50	4.45	3.44	1.67	7.18	6.54	12.81
Batch 2 Blank2	3.10	2.47	1.32	0.28	3.90	4.30	
Batch 2 Spike		113.1	85.12	62.22	57.08	44.35	

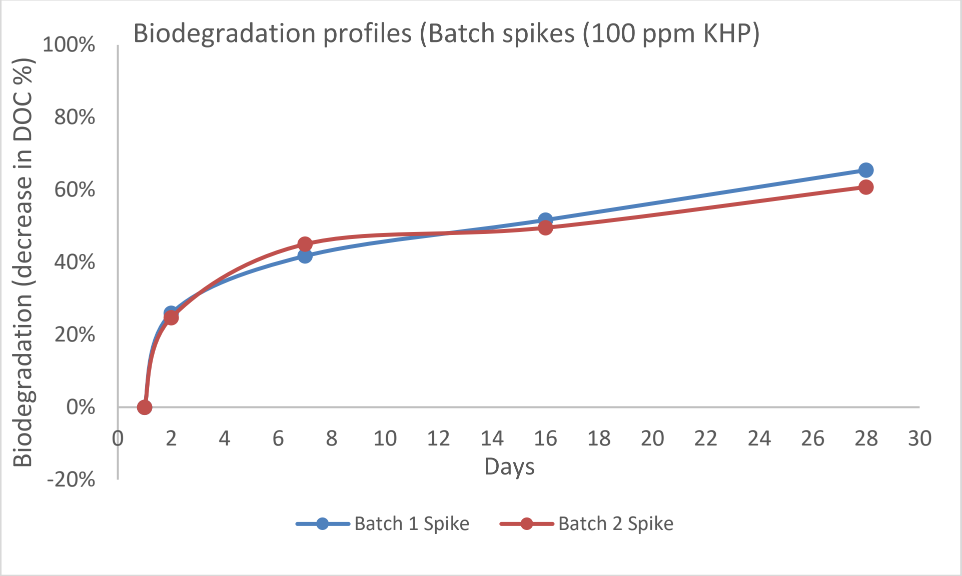


Figure 12-6 Biodegradation profile for potassium hydrogen phthalate (KHP)

Table 12-15 Final DO of samples at end of anaerobic degradation experiment

Sample ID	Replicate	DO (mg/L) after 10 d
Blank		3.19
Spike		0.86
WWTP_1 biosolids (pellets)	1	0.32
	2	0.35
	3	0.34
WWTP_1 primary sludge	1	0.63
	2	1.59
	4	0.43
WWTP_1 secondary sludge	1	0.11
	2	1.06
	3	0.37
WWTP_3 biosolids (sludge cake)	1	1.36
	2	0.34
	3	0.26
WWTP_3 secondary sludge	1	0.14
	2	0.16
	3	0.16
Reference Material (CRM 055)	1	0.05
	2	0.12
	3	0.04

Appendix 9 UV and FTIR Blanks and Reference spectra

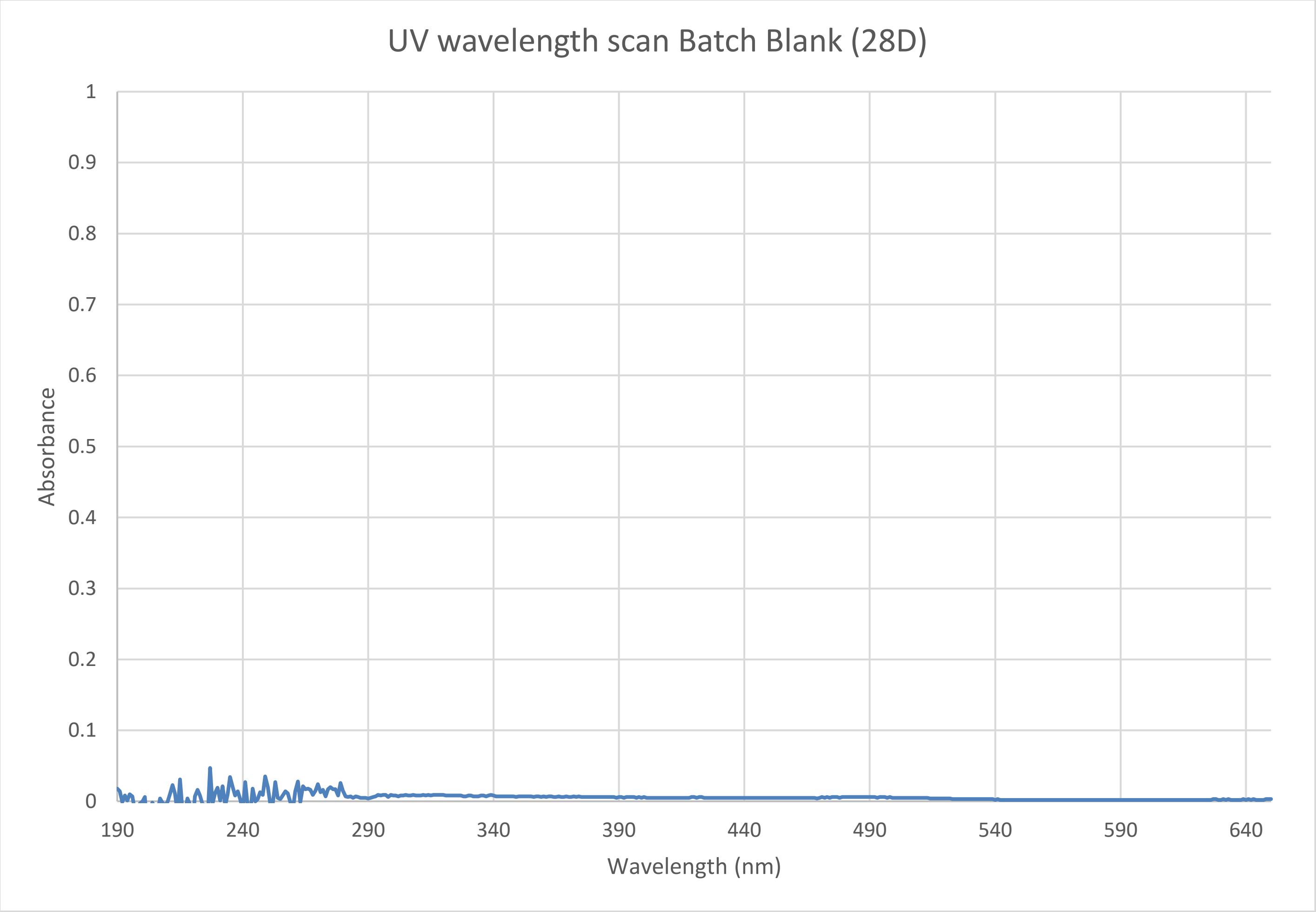


Figure 12-7 UV wavelength scan Batch Blank (28D)

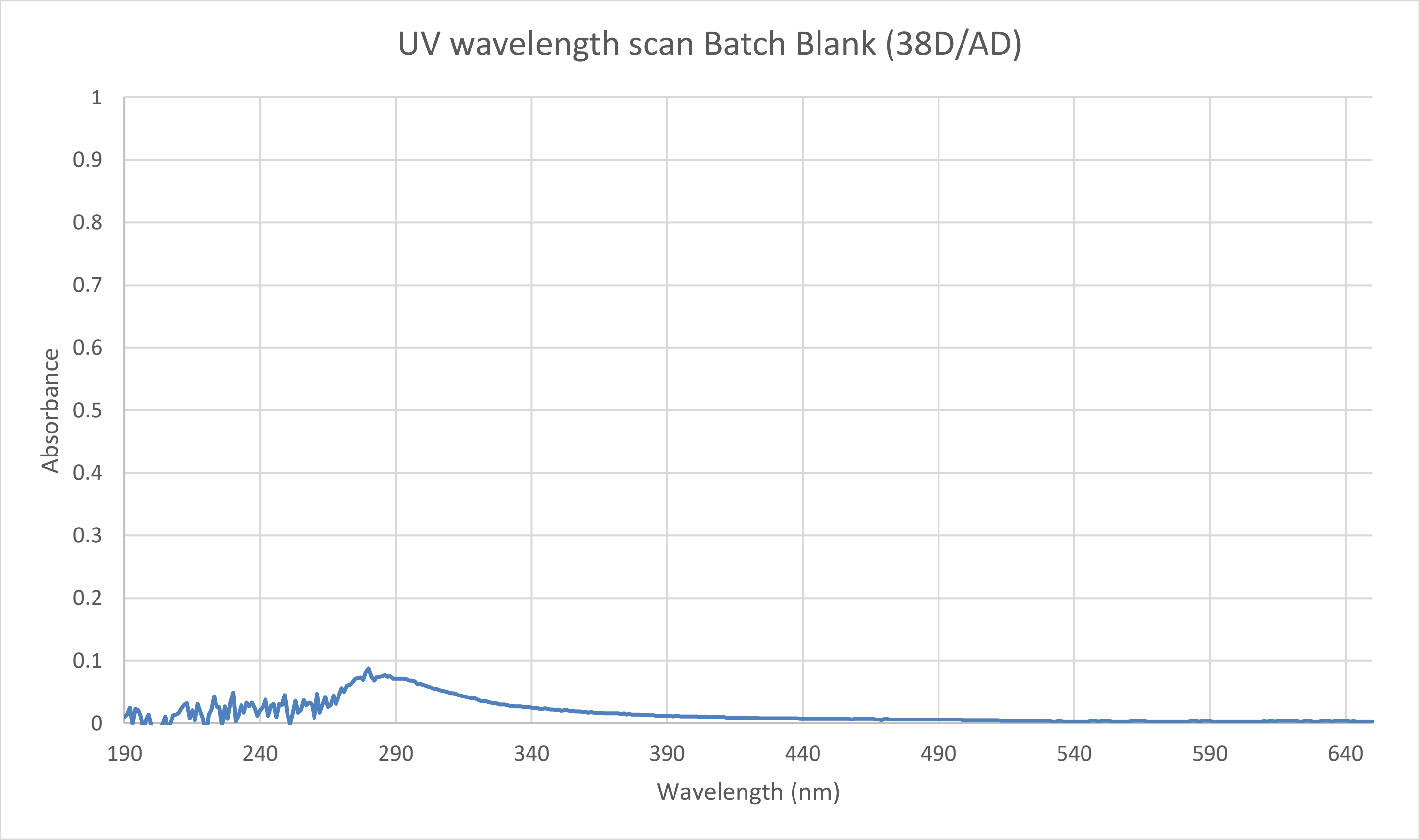


Figure 12-8 UV wavelength scan Batch Blank (38D/AD)

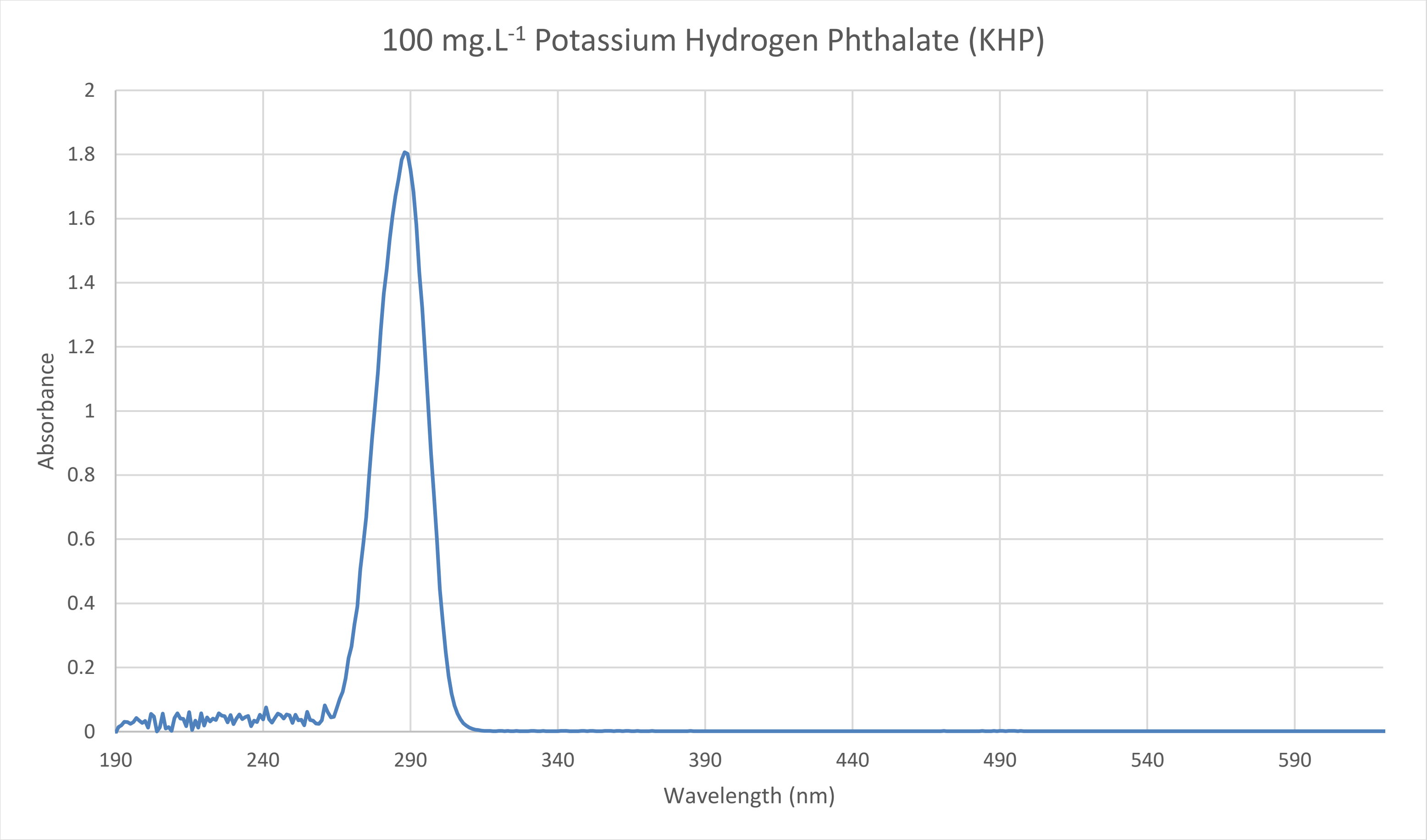


Figure 12-9 UV-Vis wavelength scan for 100 mg.L^{-1} KHP aqueous sample

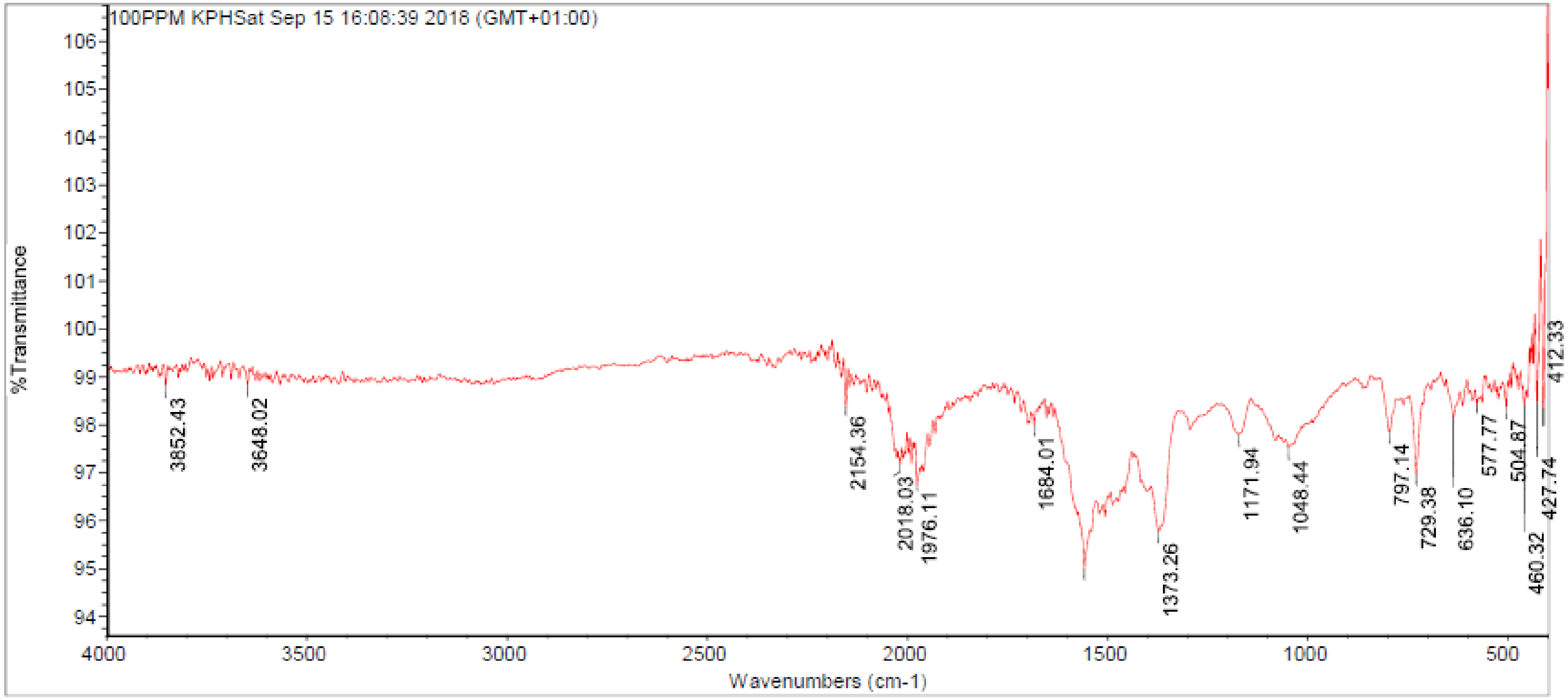
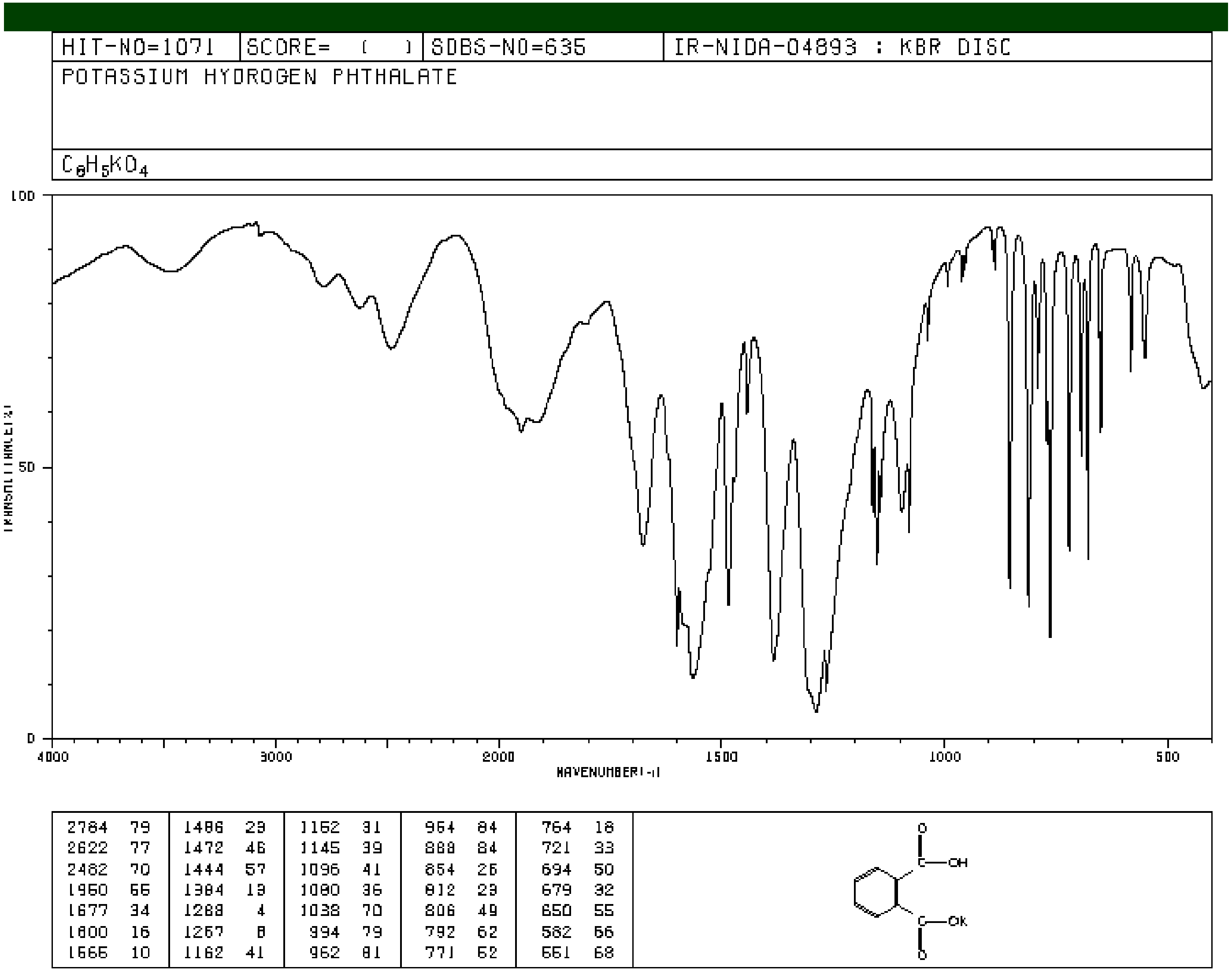


Figure 12-10 FTIR spectra for 100 mg.L^{-1} Potassium Hydrogen Phthalate (KHP) aqueous solution prepared for this study.



HIT-NO=1071	SCORE= ( )	SDBS-NO=635	IR-NIDA-04893 : KBR DISC

POTASSIUM HYDROGEN PHTHALATE

$C_8H_5KO_4$

2784	79	1486	29	1162	31	964	84	764	18
2622	77	1472	46	1145	39	888	84	721	33
2482	70	1444	57	1096	41	854	26	694	50
1960	66	1384	13	1080	36	812	29	679	32
1677	34	1288	4	1038	70	806	49	650	55
1800	16	1267	8	994	79	792	62	582	66
1666	10	1162	41	962	81	771	62	661	68

Figure 12-11 FTIR Reference spectra for potassium hydrogen phthalate (SDBS 2018).

A comparison of the FTIR frequencies of sample KHP (Figure 12-10) and reference KHP (Figure 12-11) find agreement, albeit peak shifts owing to the variation in source materials. A summary of key peak assignments is presented in Table 12-16. O-H carboxylic stretching in the 3200-2500 wavelengths, and aromatic out of plan bending were not observed in the KHP sample compared to the reference, owing to the reduced sample volume.

Table 12-16 FTIR frequency assignment of reference and sample KHP

Peak assignment	Reference KHP	Sample KHP
O-H carboxylic stretching	2784, 2622, 2482	
Double bond asymmetric stretch	1960	1976
Double bond stretch	1677	1684
C-OH carboxylic stretch	1384	1373
C-O carboxylic stretch	1162, 1152, 1145	1172
Inorganics and minerals	1038	1048
Aromatic benzene (out of plane bending)	994, 962, 954, 888	
C=C bending	792	797

References

Spectral Database for Organic Compounds (SDBS). 2018. Potassium Hydrogen Phthalate SDBS No. 635. [online] Available at: https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi]. [Accessed 15 Sept. 2018].

Simonescu, C. M. 2012. Application of FTIR Spectroscopy in Environmental Studies. In: Farrukh, A.M. (Ed.), Advanced Aspects of Spectroscopy, Intech Publishers, New York, pp. 49-84.

Appendix 10 FTIR Spectra and Replicates

12.10.1 Spectra for WWTP dried solids (Primary, Secondary sludges and Treated Biosolids)

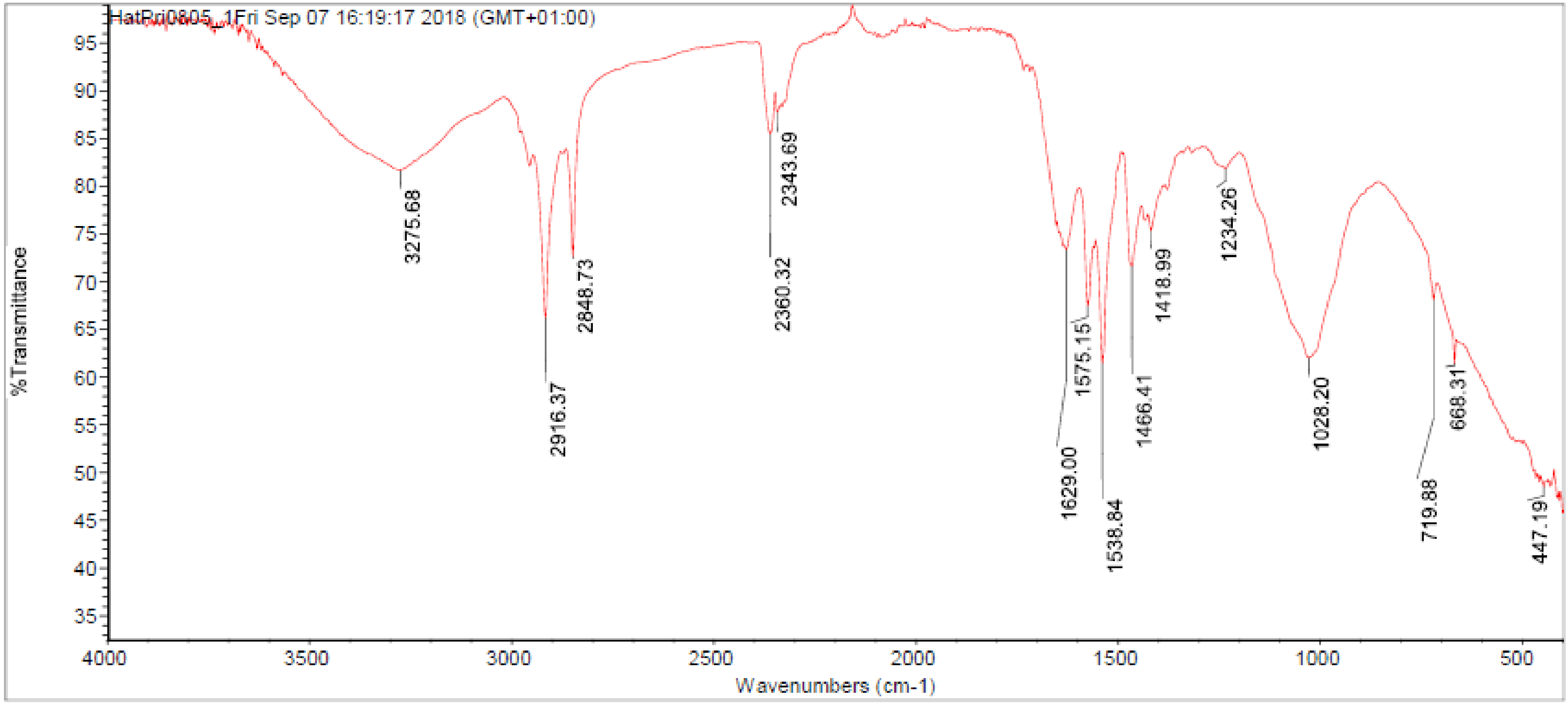


Figure 12-12FTIR Spectra WWTP_1_Pri (May) dried solid (Rep 1)

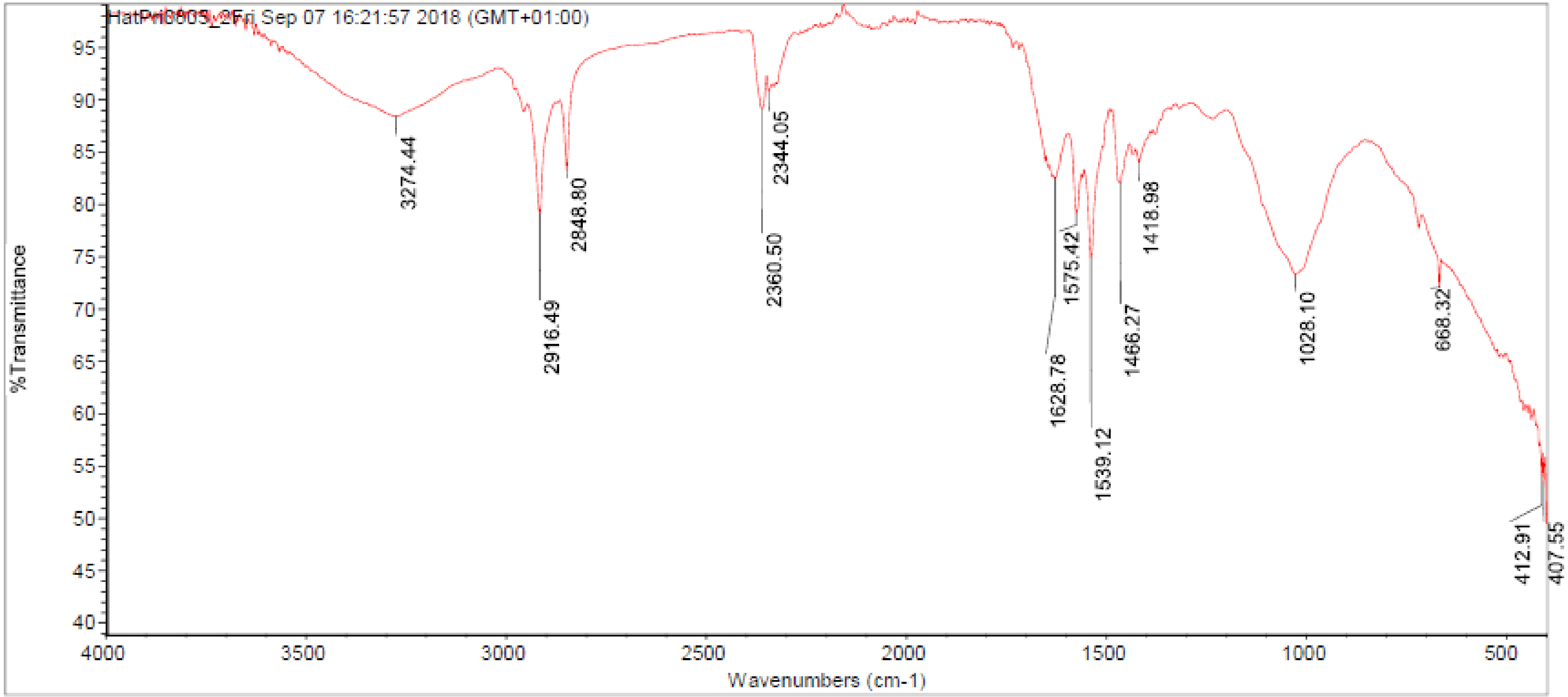


Figure 12-13 FTIR Spectra WWTP_1_Pri (May) dried solid Rep (2)

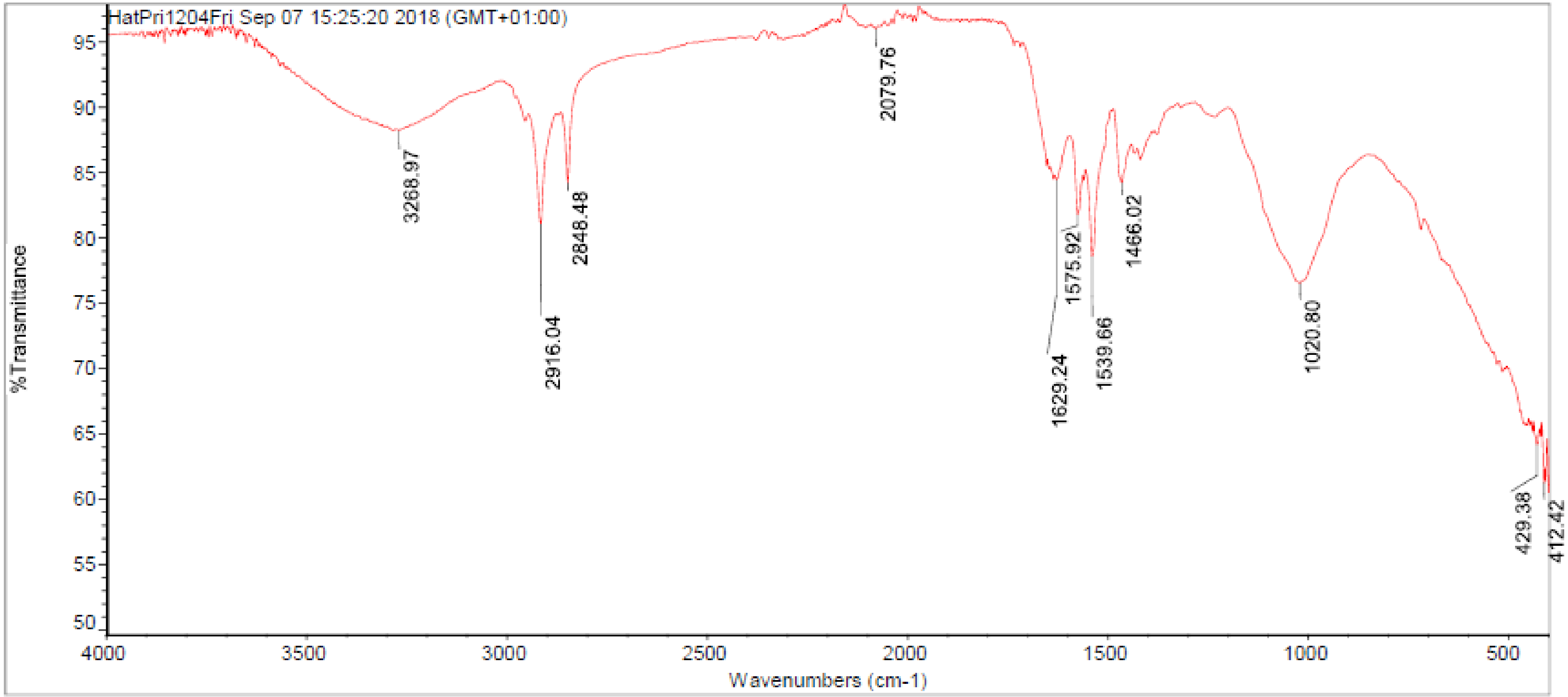


Figure 12-14 FTIR Spectra WWTP_1_Pri (Apr) dried solid Rep 1

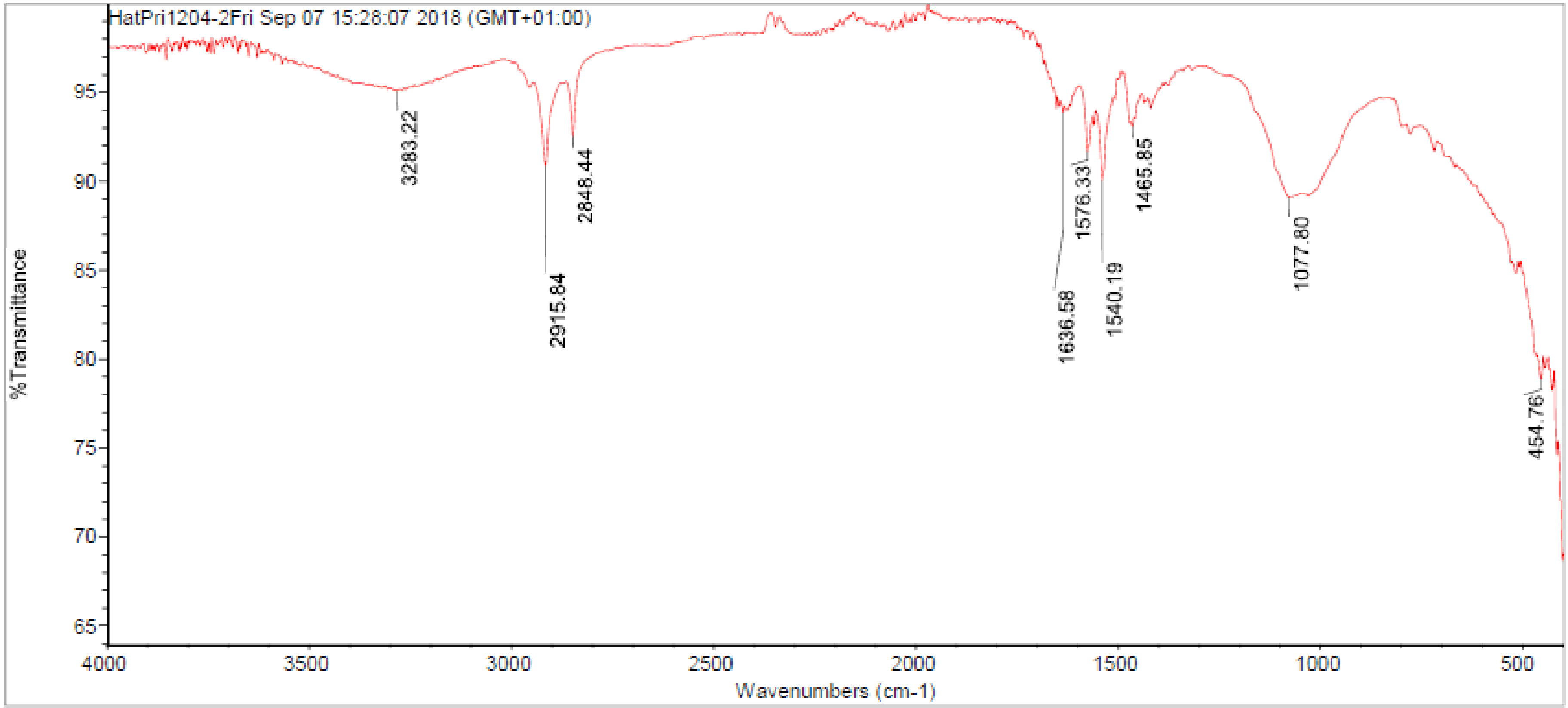


Figure 12-15 FTIR Spectra WWTP_1_Pri (Apr) dried solid (Rep 2)

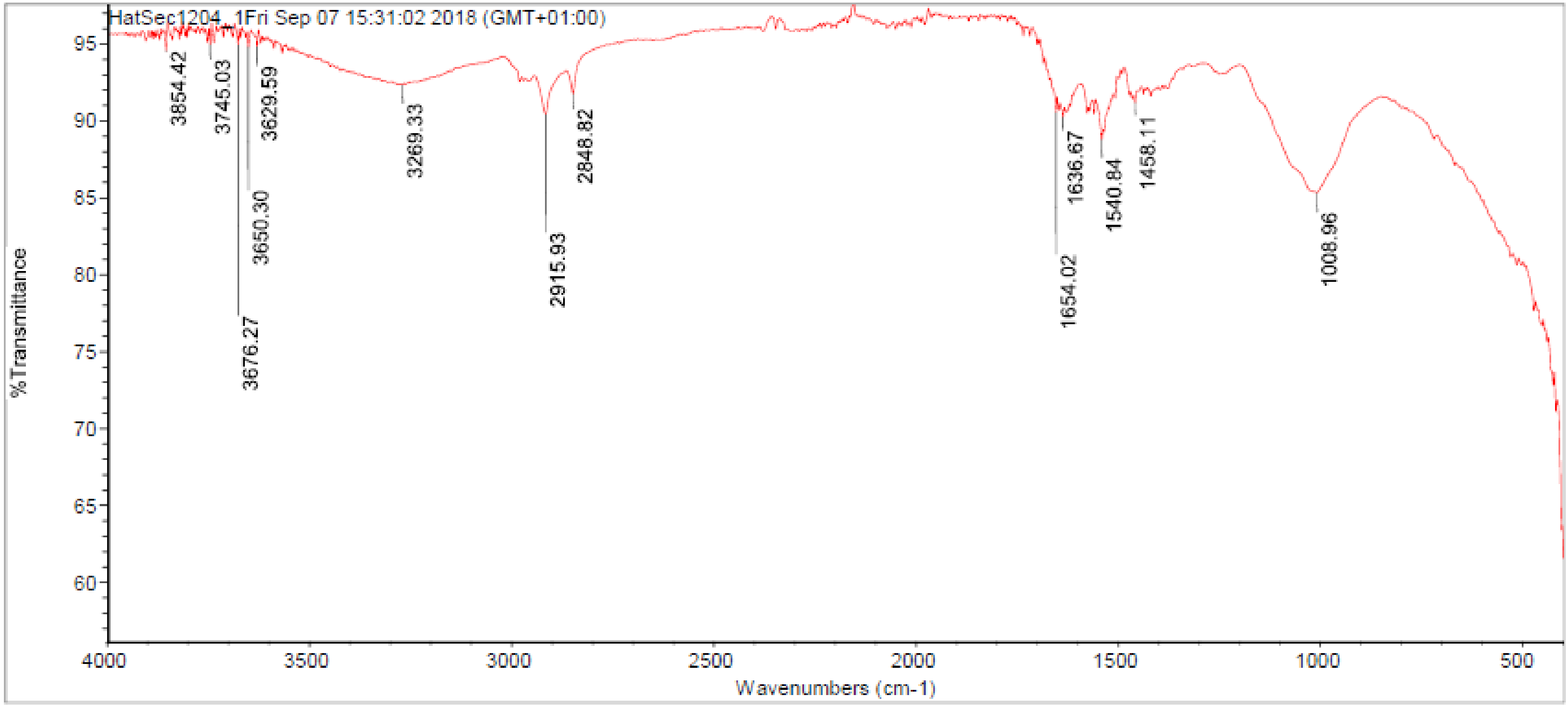


Figure 12-16 FTIR Spectra WWTP_1_Sec (Apr) dried solid

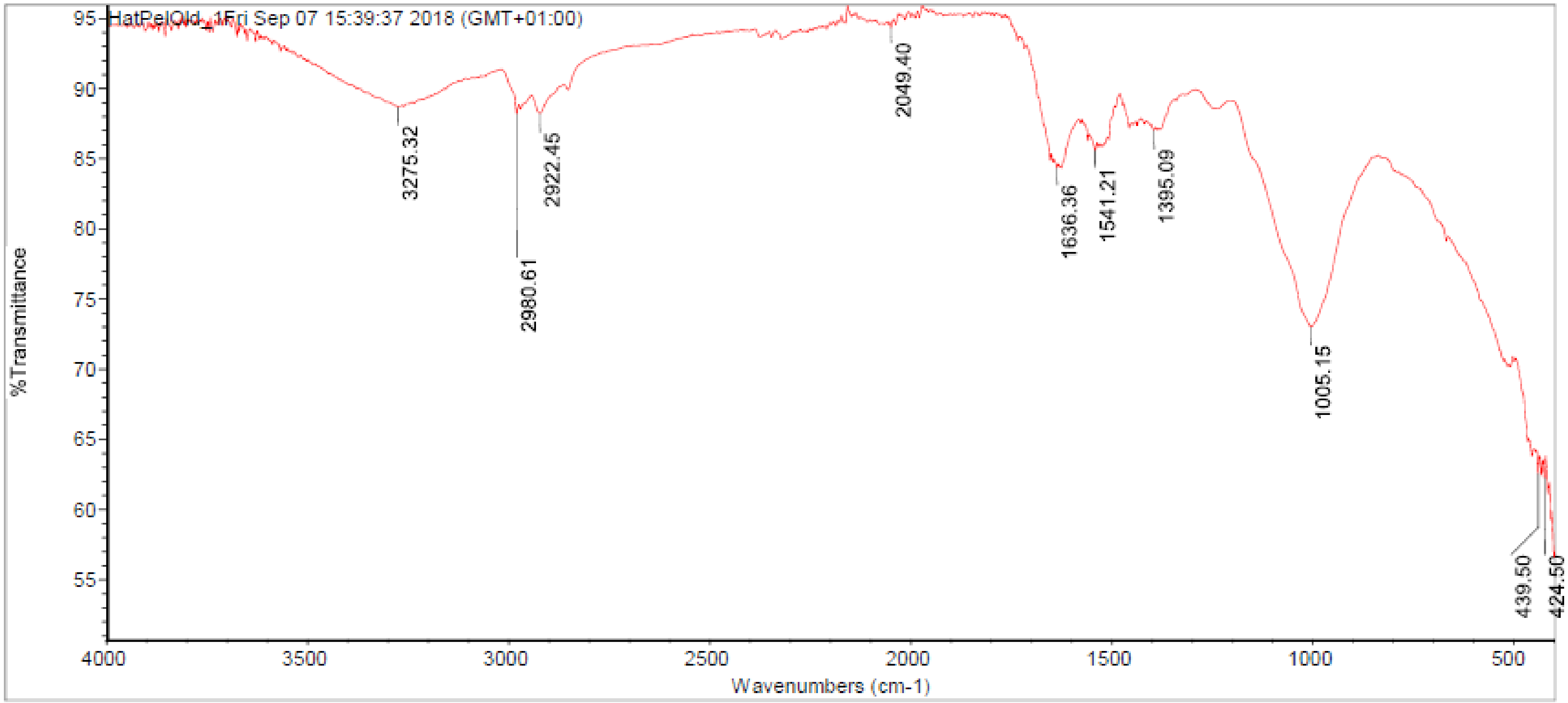


Figure 12-17 FTIR Spectra WWTP_1_Biosolid_old , dried solid (Rep 1)

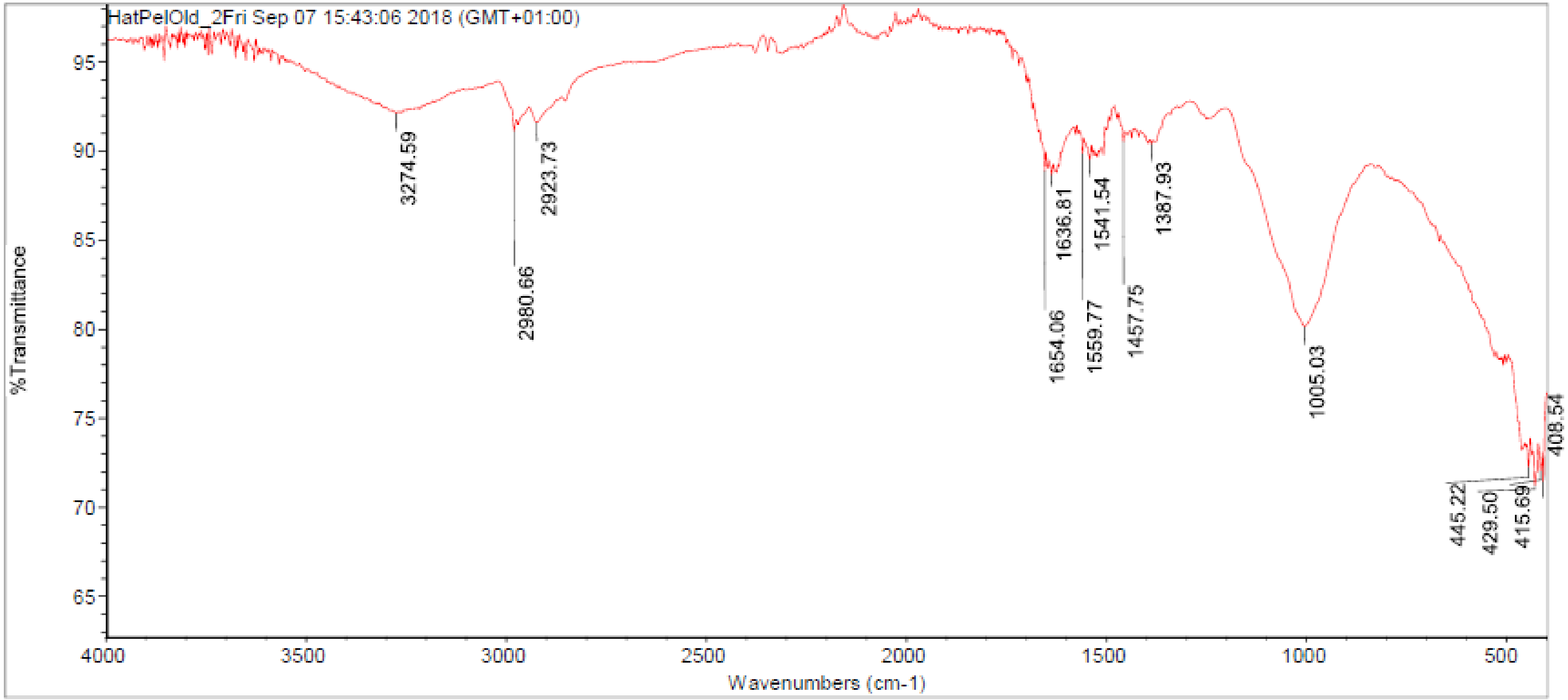


Figure 12-18 FTIR Spectra WWTP_1_Biosolid_old dried solid (Rep 2)

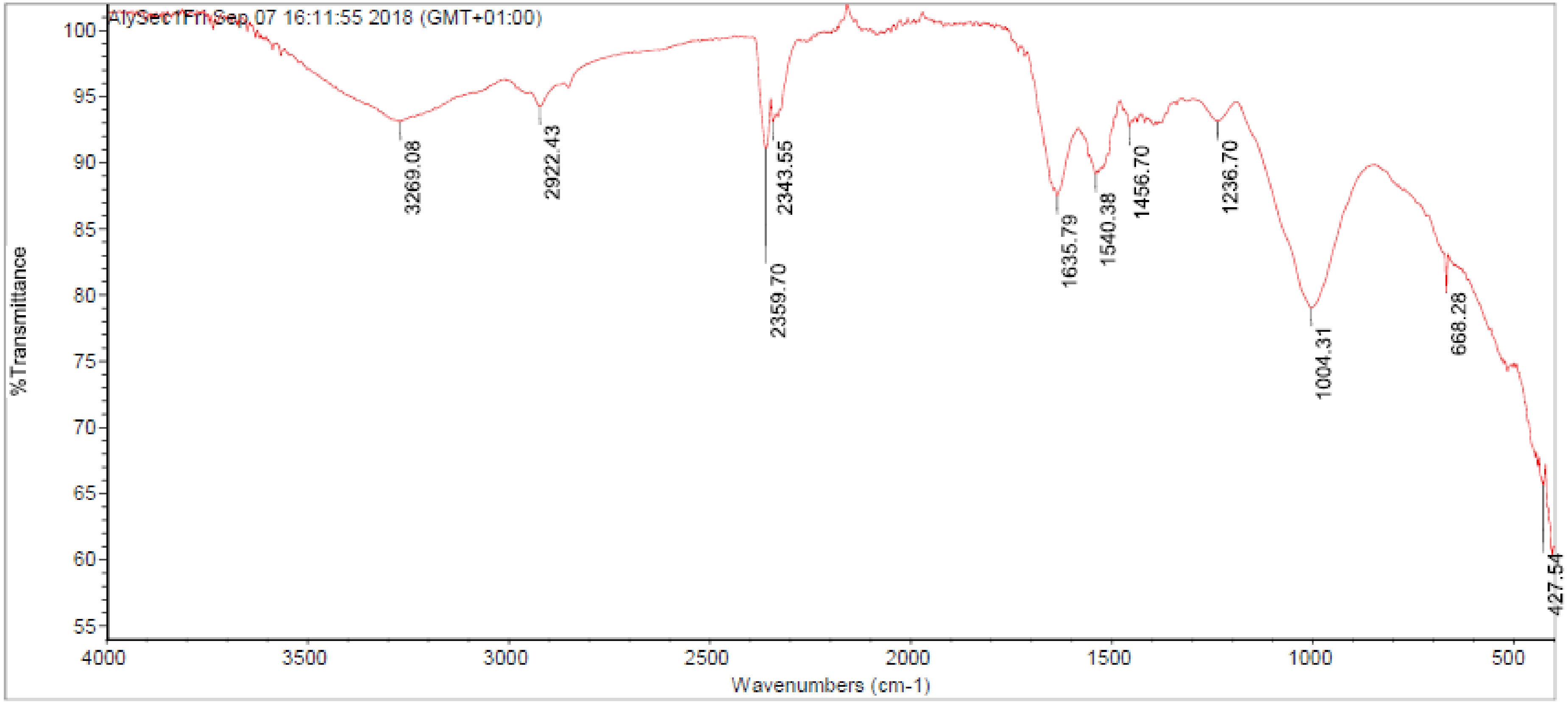


Figure 12-19 FTIR Spectra WWTP_2_Secondary sludge dried solid

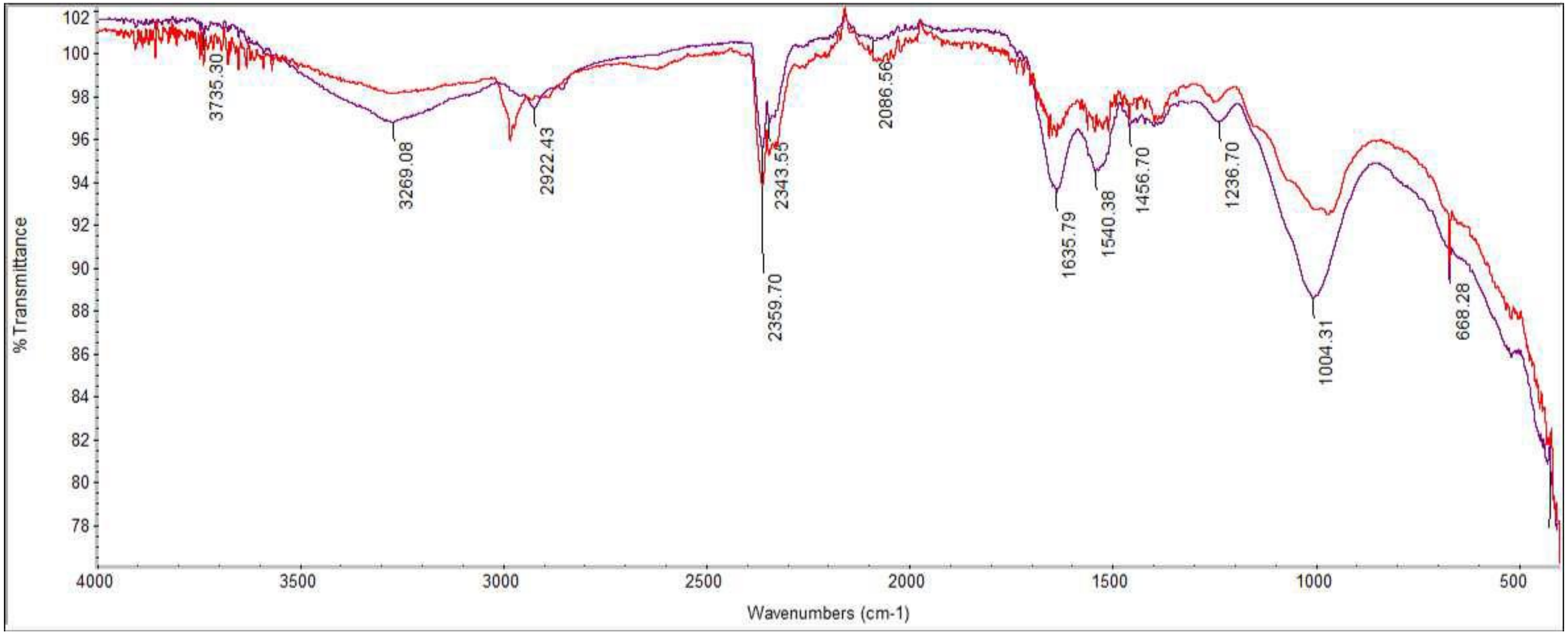


Figure 12-20 FTIR Spectra WWTP_2_Secondary sludge dried solid (Rep 1 and 2 overlay)

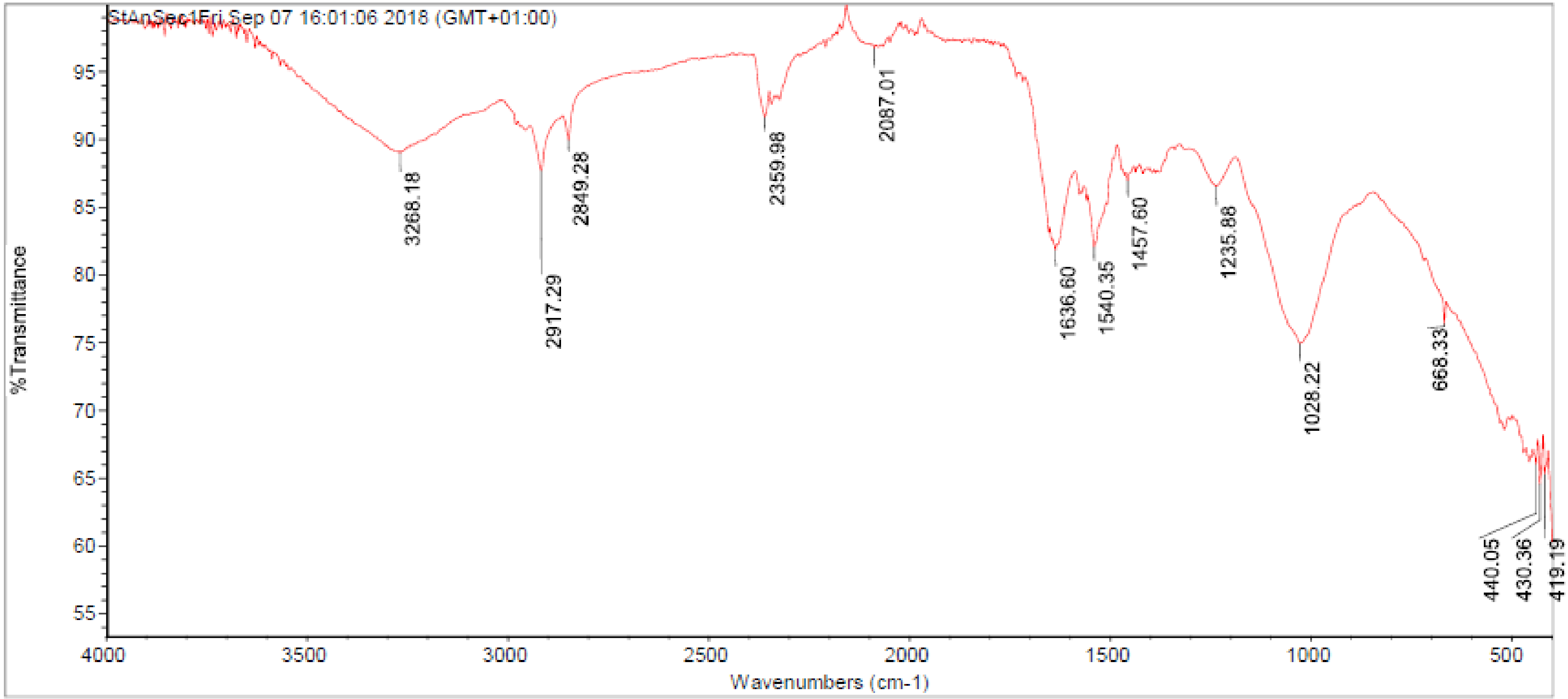


Figure 12-21 FTIR Spectra WWTP_3_Secondary sludge dried solid

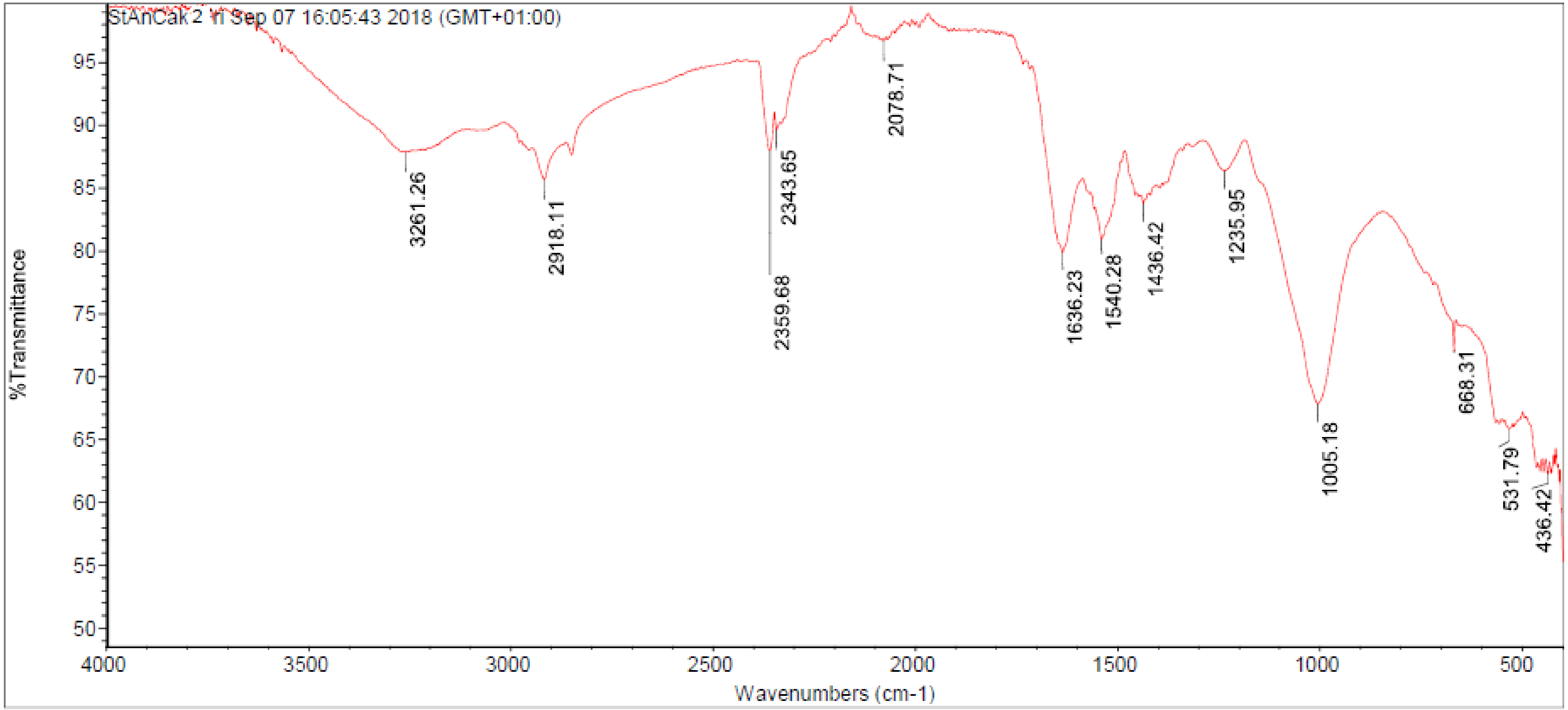


Figure 12-22 FTIR Spectra WWTP_3_treated biosolid, dried solid

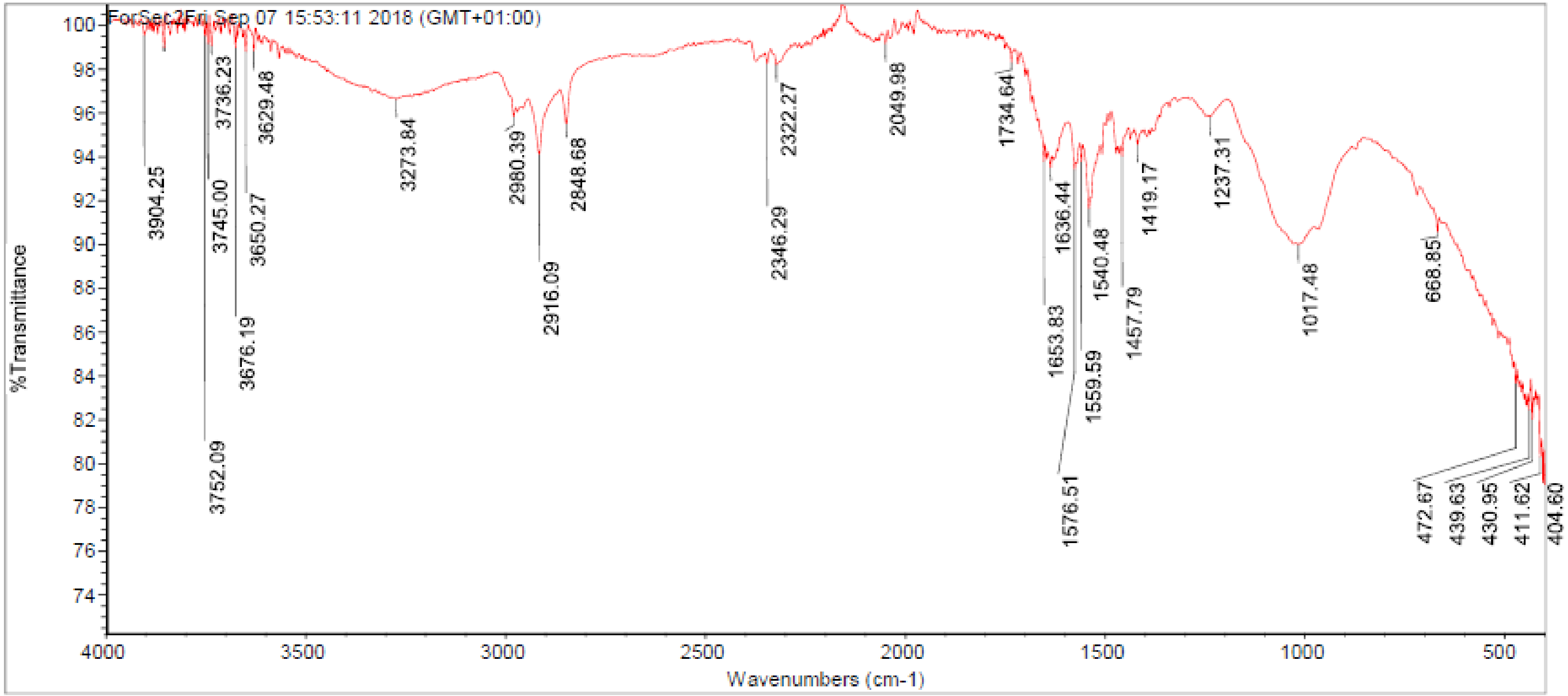


Figure 12-23 FTIR Spectra WWTP_4_Secondary sludge, dried solid

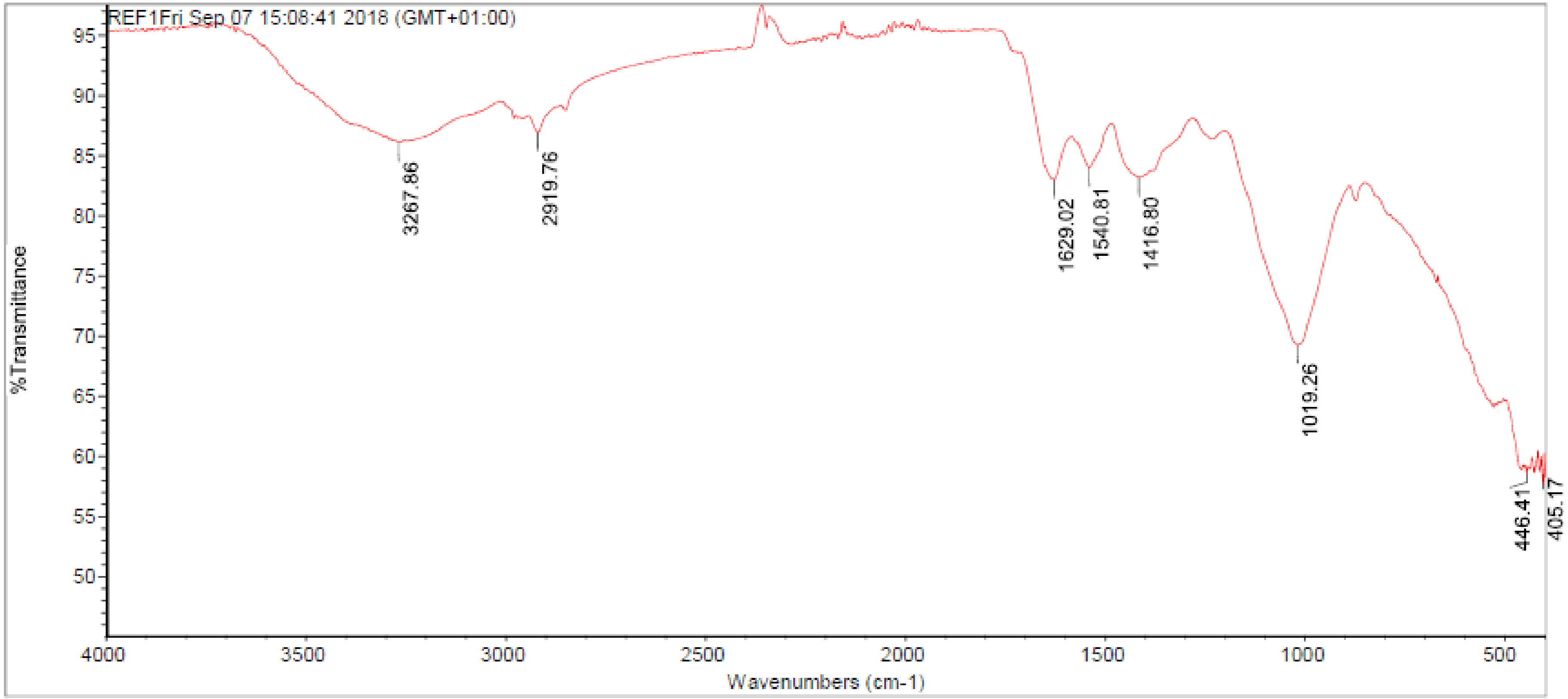


Figure 12-24 FTIR Spectra Reference Material treated biosolid, dried solid (Rep 1)

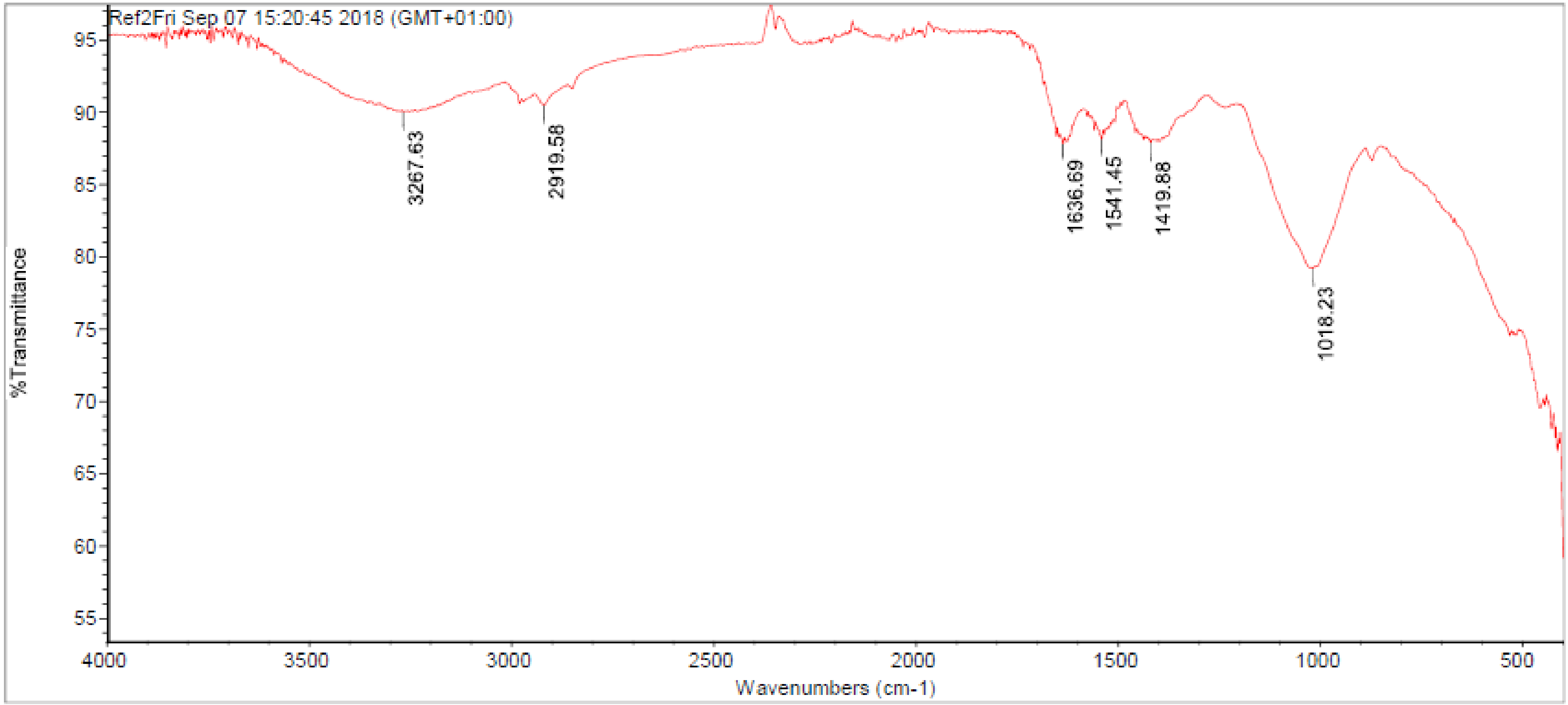
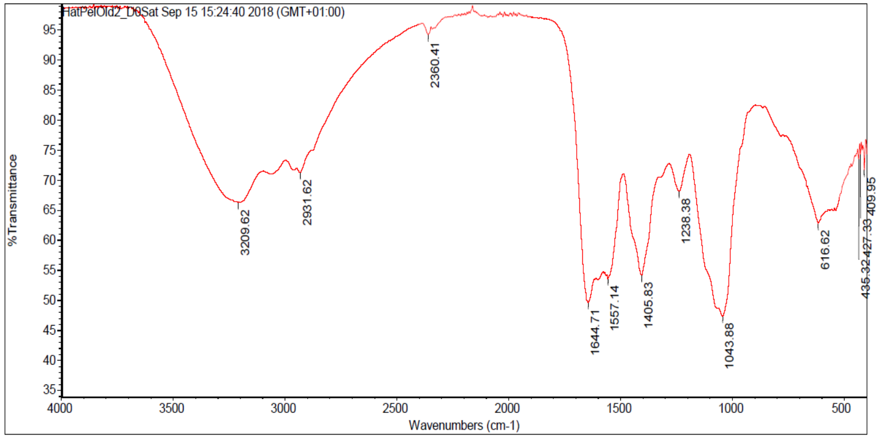
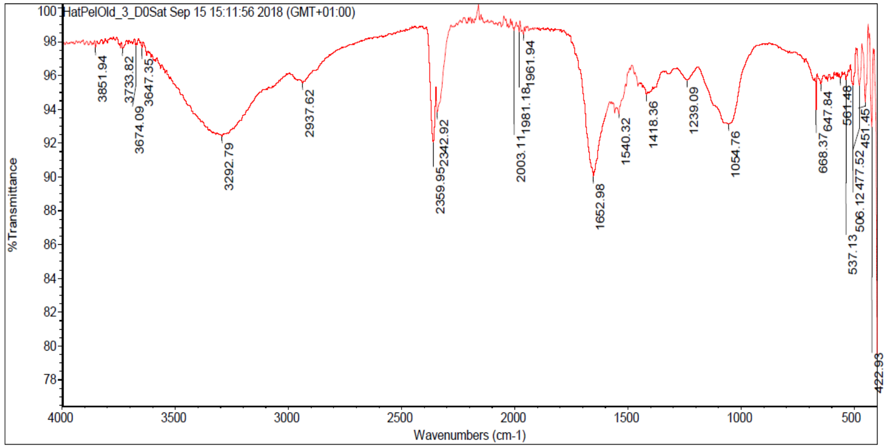


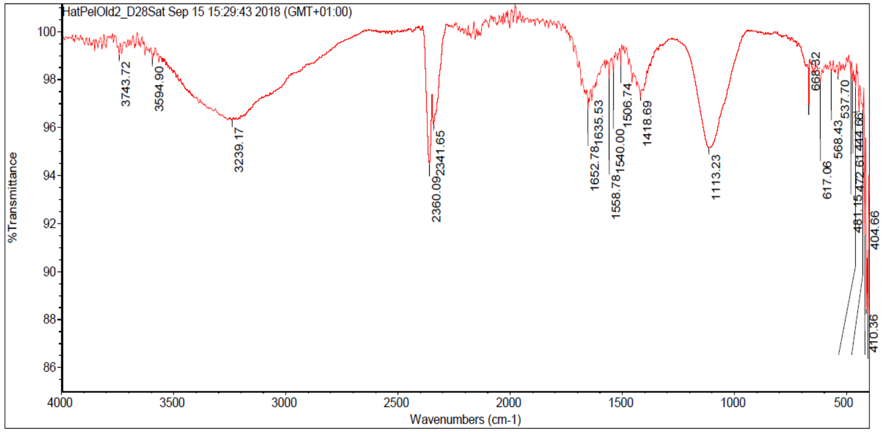
Figure 12-25 FTIR Spectra Reference Material treated biosolid, dried solid (Rep 2)

12.10.2 FTIR Spectra for liquid leachates

WWTP_1_Treated Biosolids (Rep 1)	WWTP_1_Treated Biosolids (Rep 2)
D0 	D0 

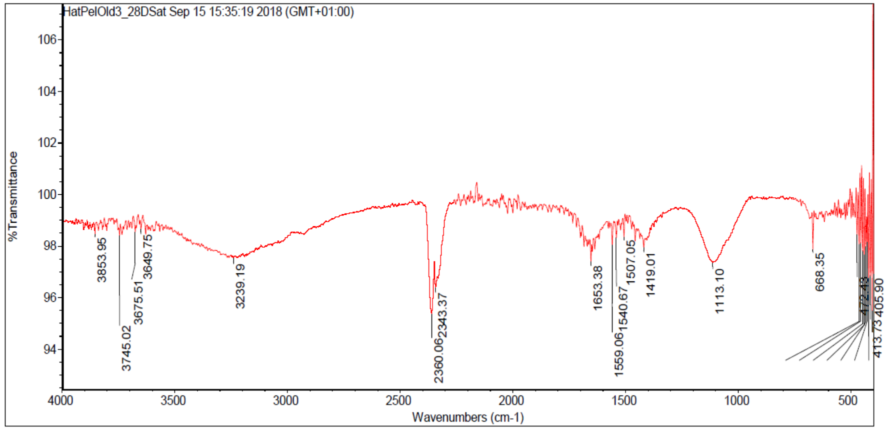
WWTP_1_Treated Biosolids (Rep 1)

D28



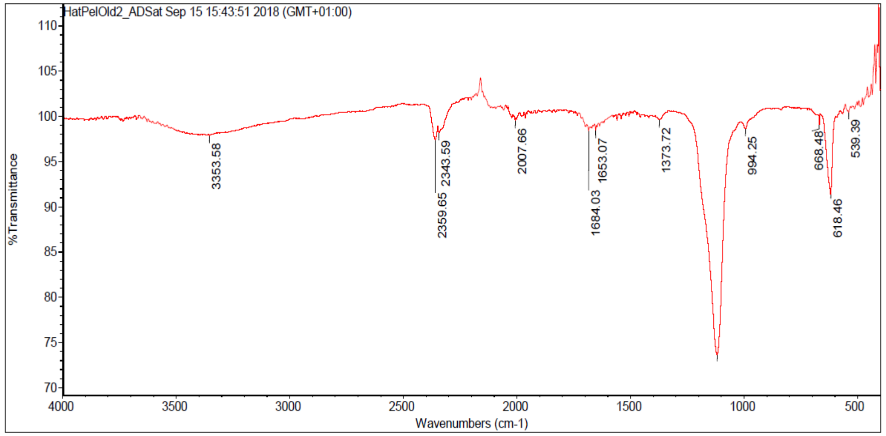
WWTP_1_Treated Biosolids (Rep 2)

D28



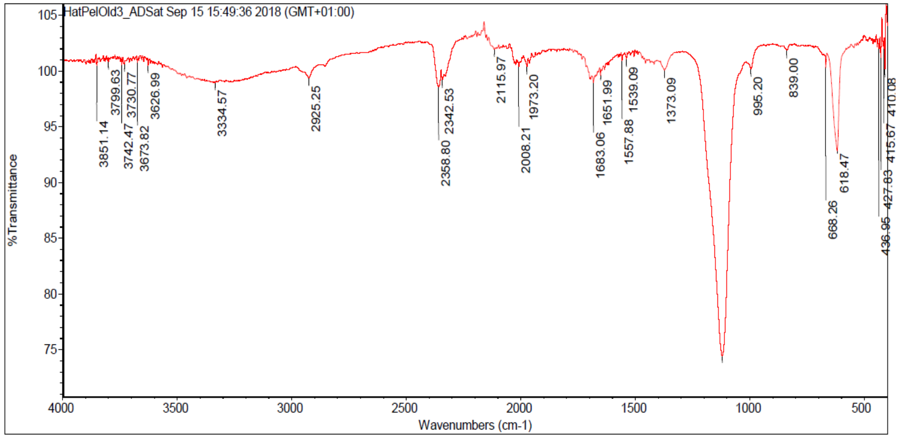
WWTP_1_Treated Biosolids (Rep 1)

D38



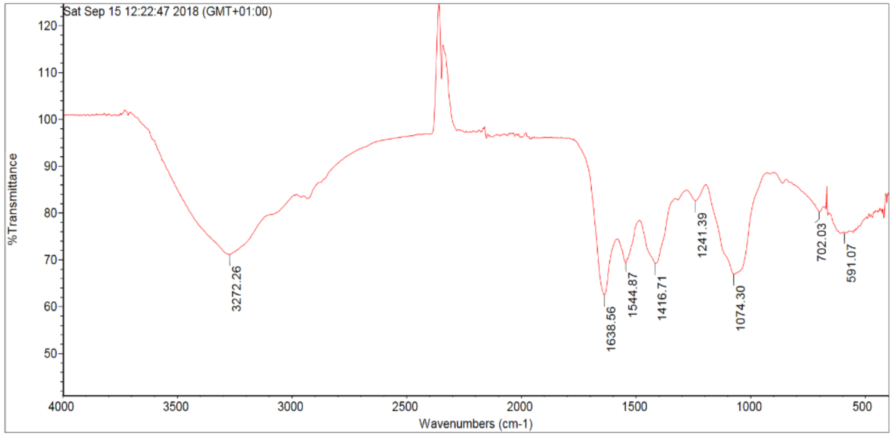
WWTP_1_Treated Biosolids (Rep 2)

D38



WWTP_1_Primary (May) (Rep 1)

D0



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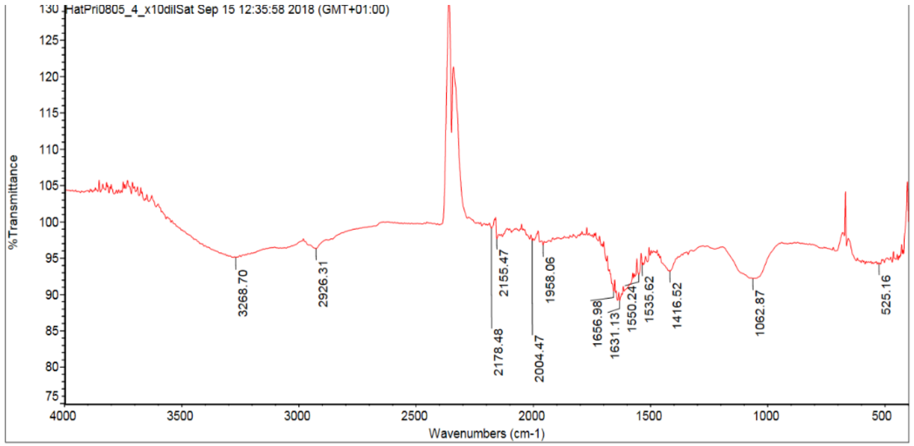
D0



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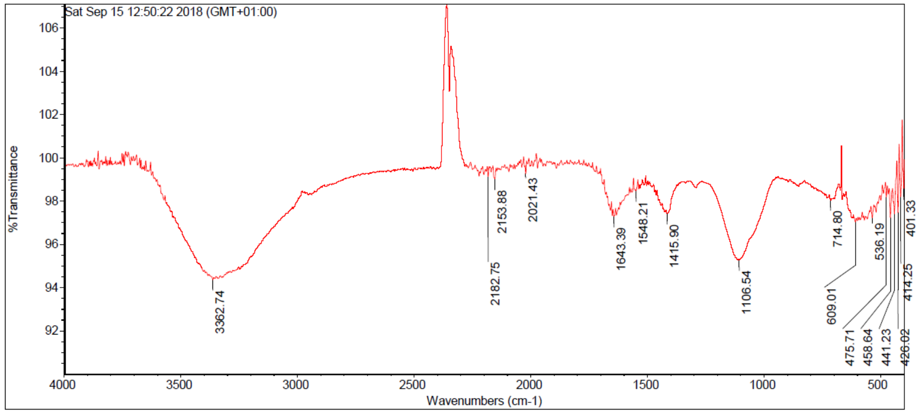

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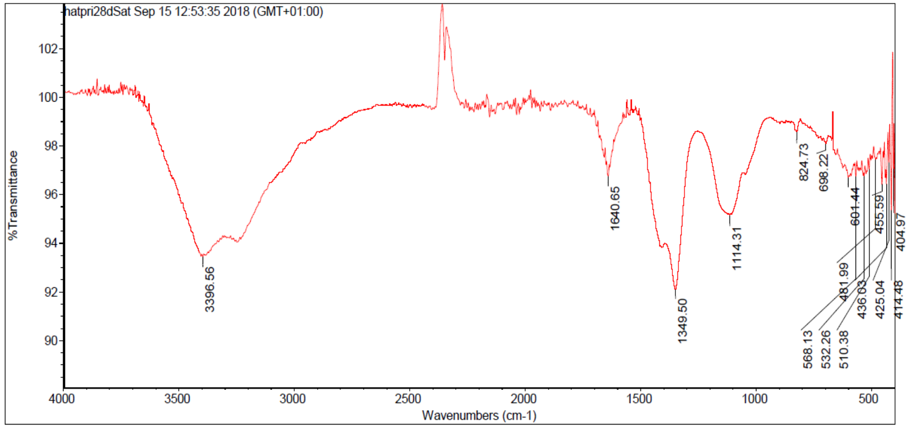

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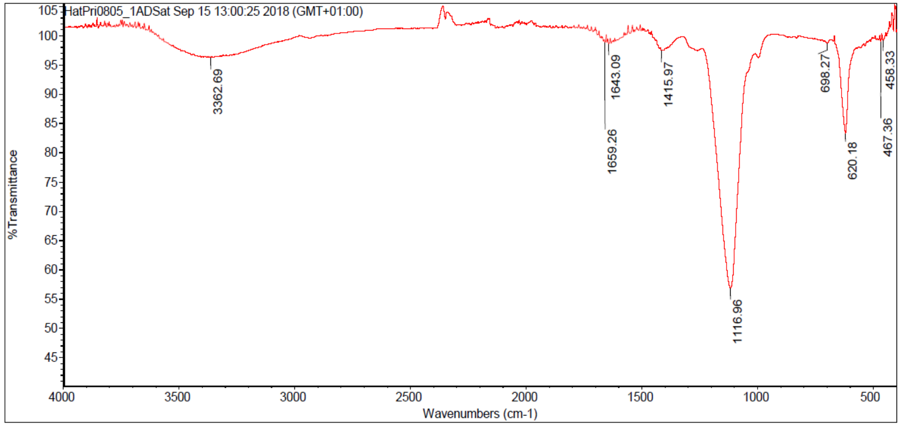
AD



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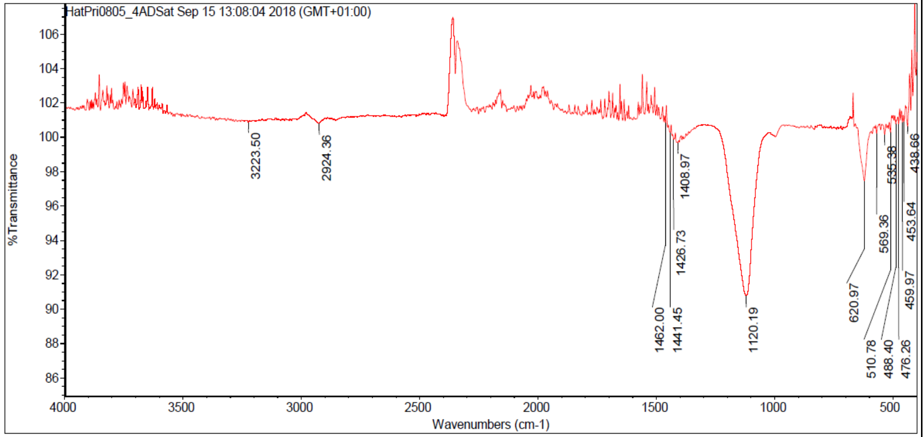

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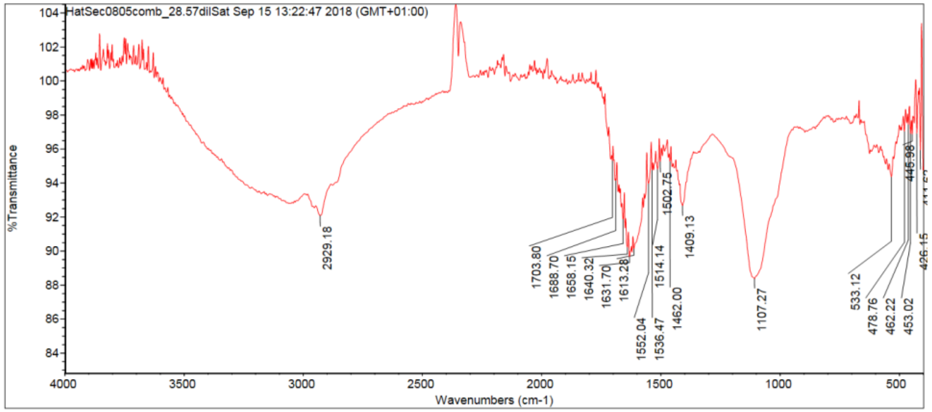
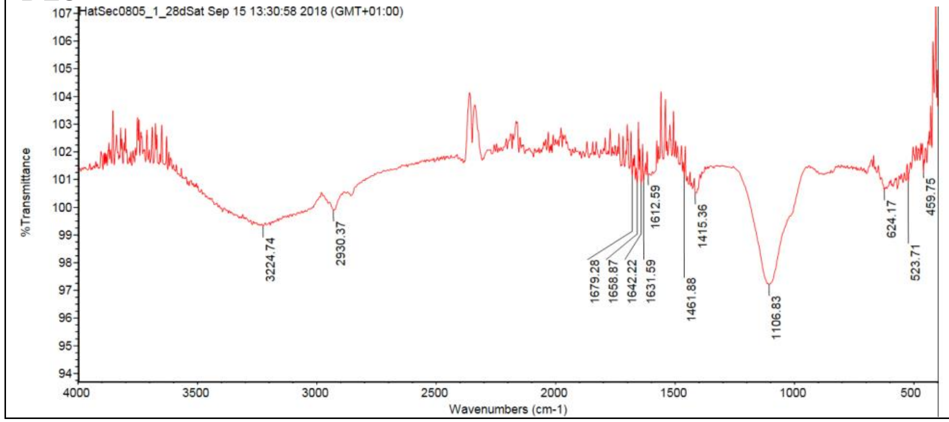


*Only 1 replicate analysed for D0

WWTP_1_Secondary (May) (Rep 1)

D28



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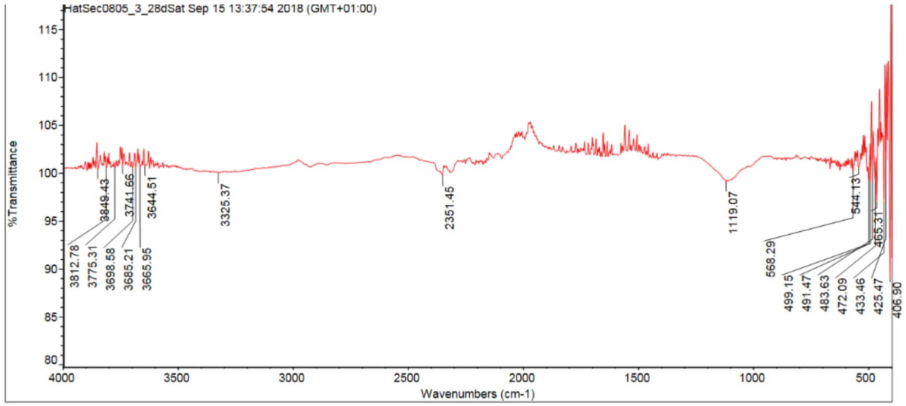

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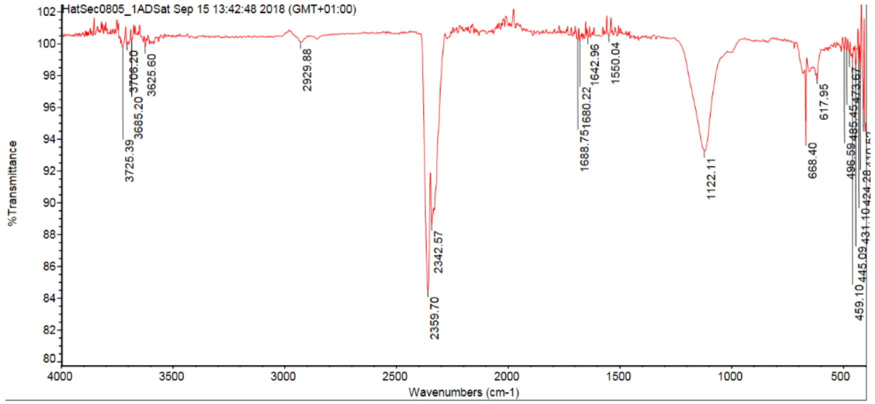

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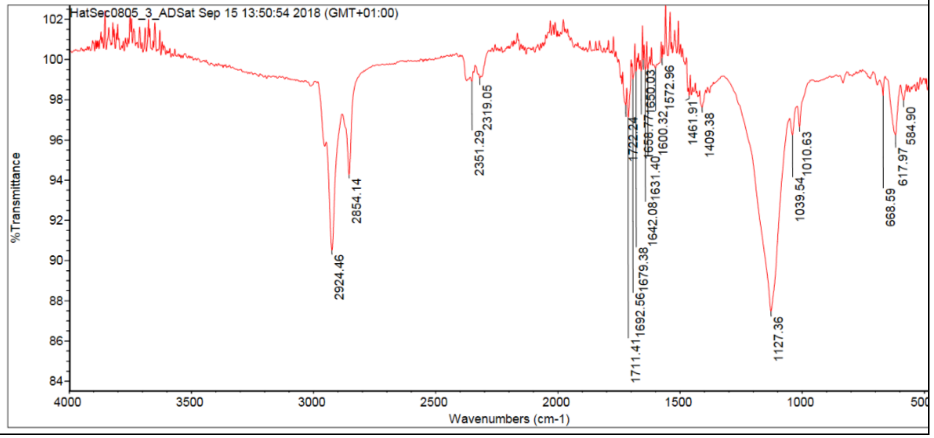
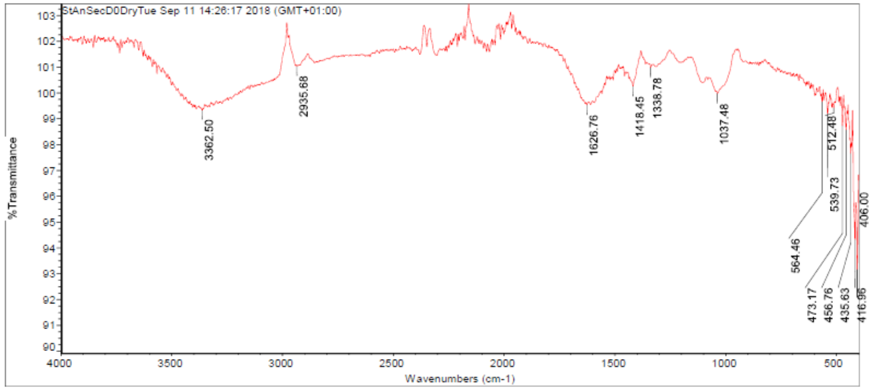
D38



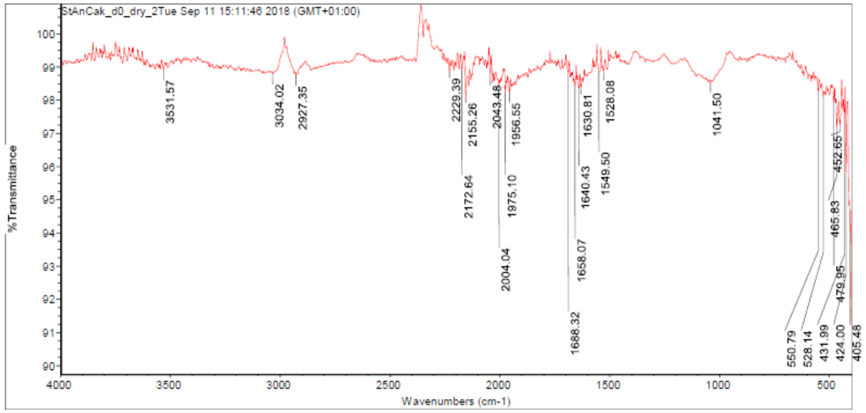
WWTP_3 Secondary Sludge

D0



WWTP_3 Sludge Cake

D0



WWTP_3 Secondary Sludge

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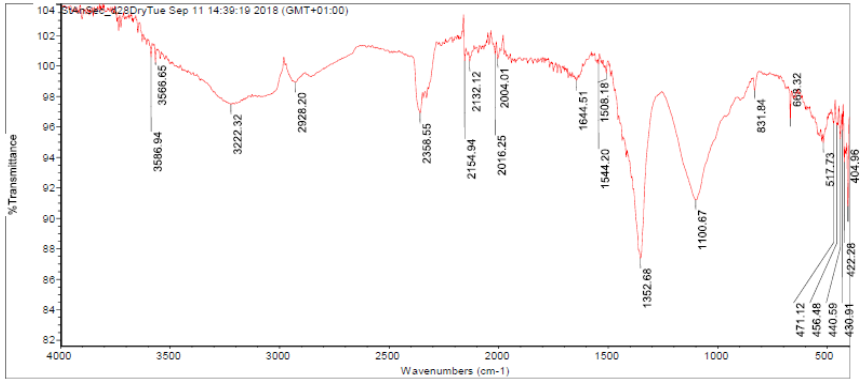
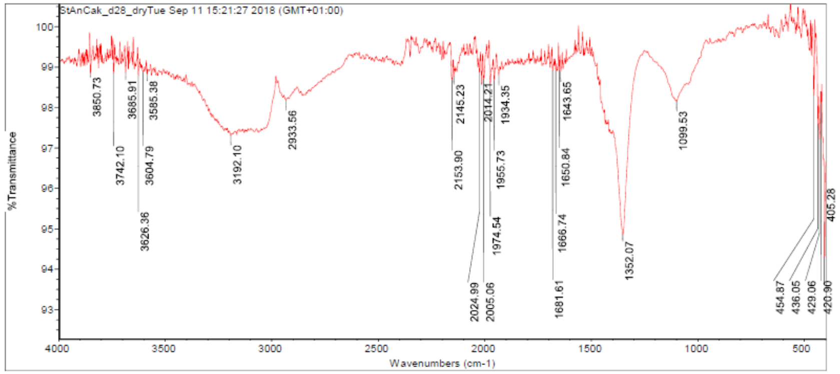


WWTP_3 Sludge Cake

D28



WWTP_3 Secondary Sludge

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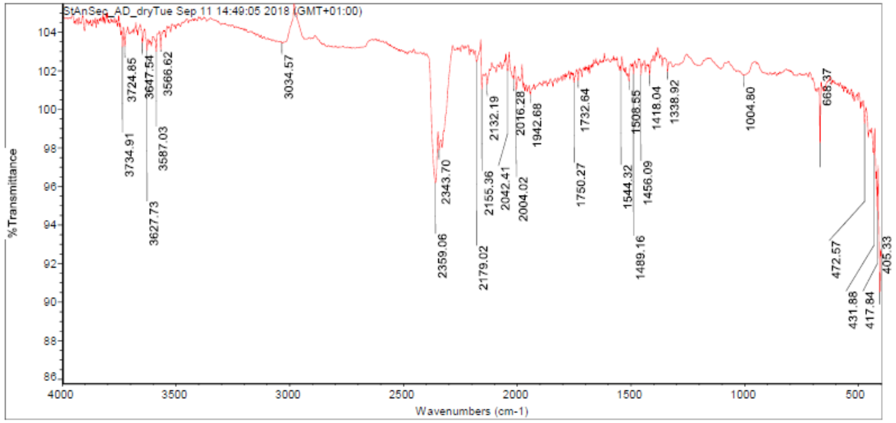

WWTP_3 Sludge Cake

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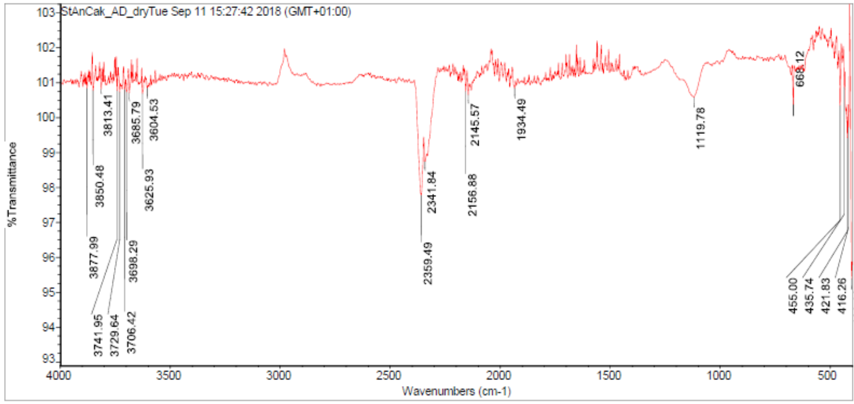
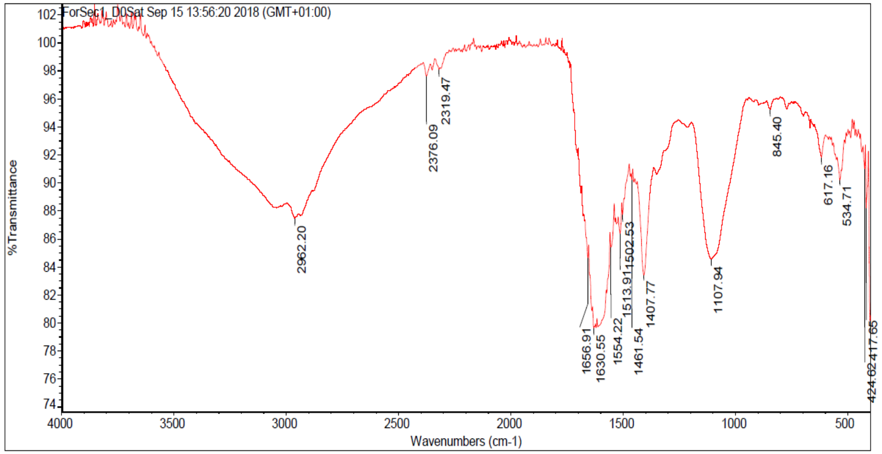


WWTP_4_Secondary sludge (Rep 1)

D0



WWTP_4_Secondary sludge (Rep 2)

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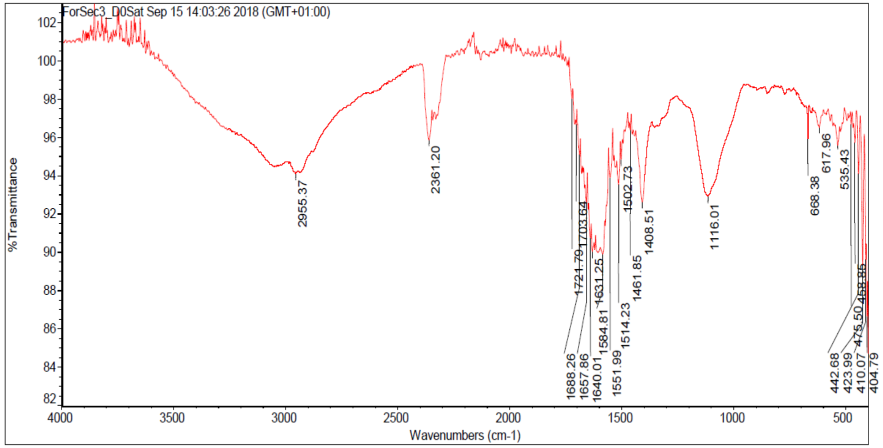

WWTP_4_Secondary sludge (Rep 1)

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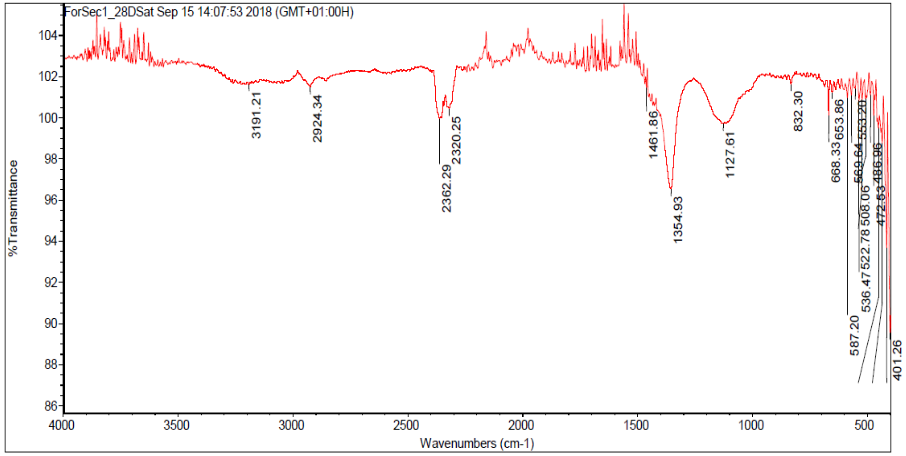

WWTP_4_Secondary sludge (Rep 2)

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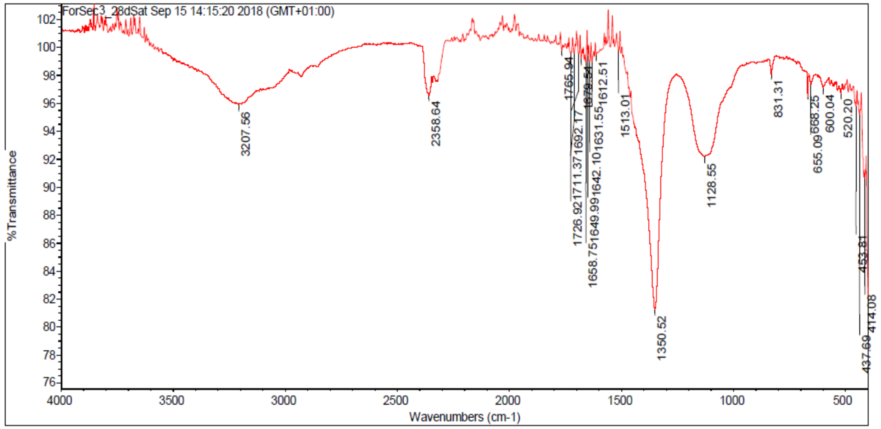
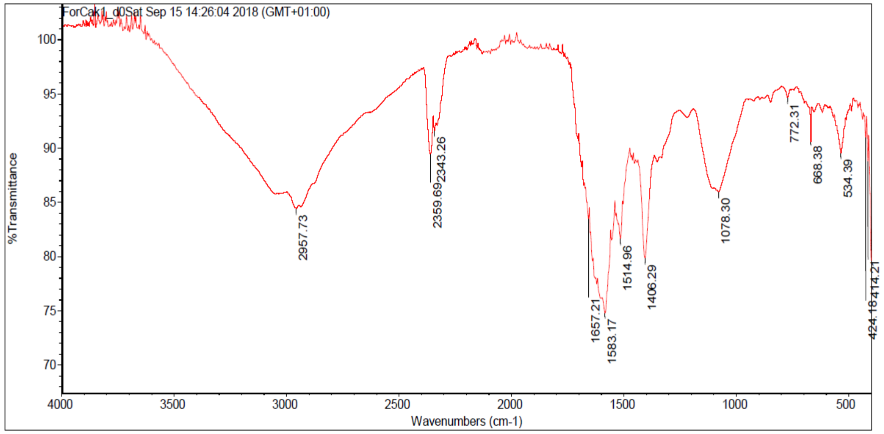


WWTP_4 Sludge Cake (Rep 1)
D0



WWTP_4 Sludge Cake (Rep 2)
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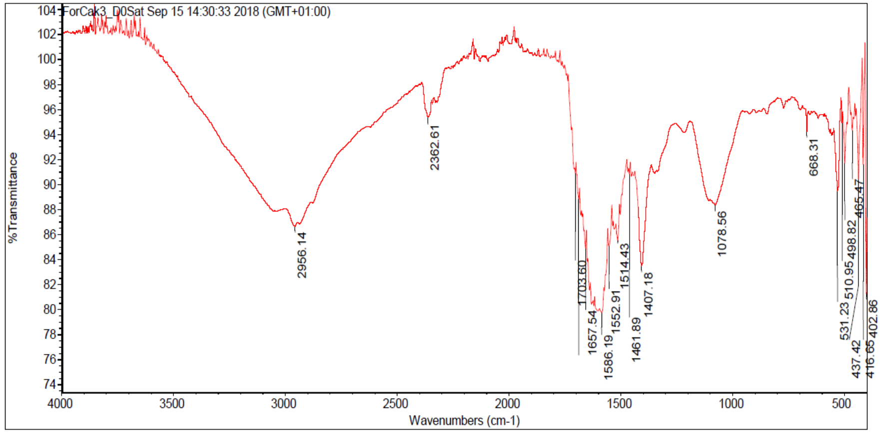

Reference Material (Rep 1)

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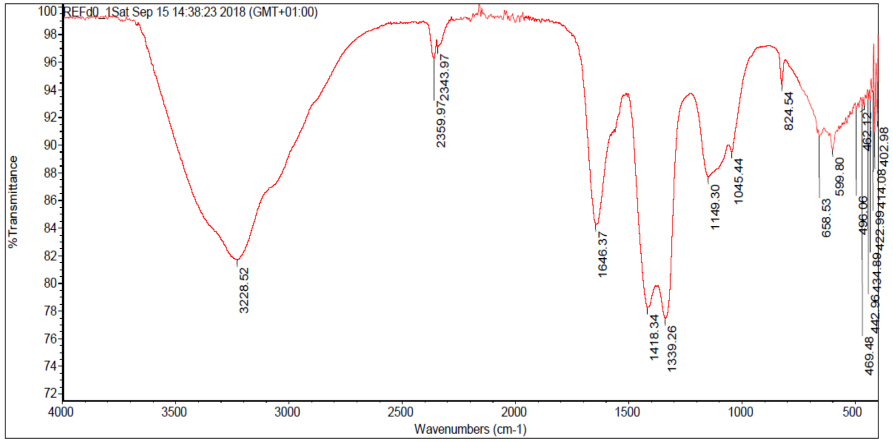

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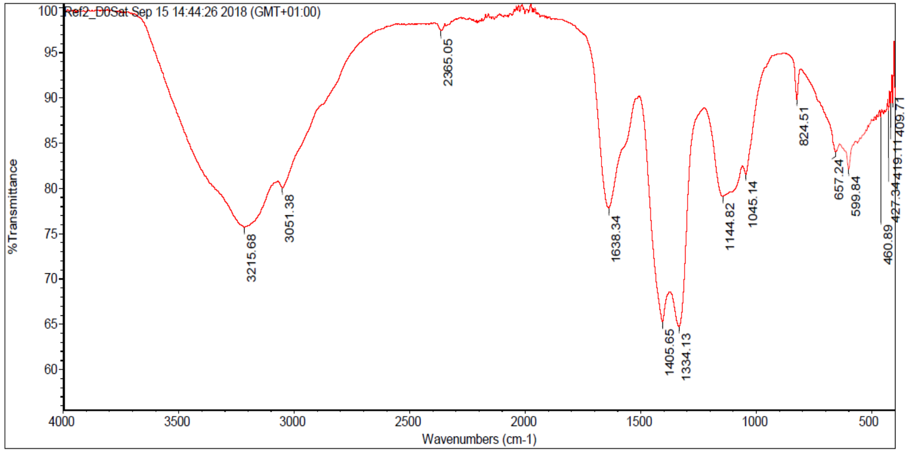

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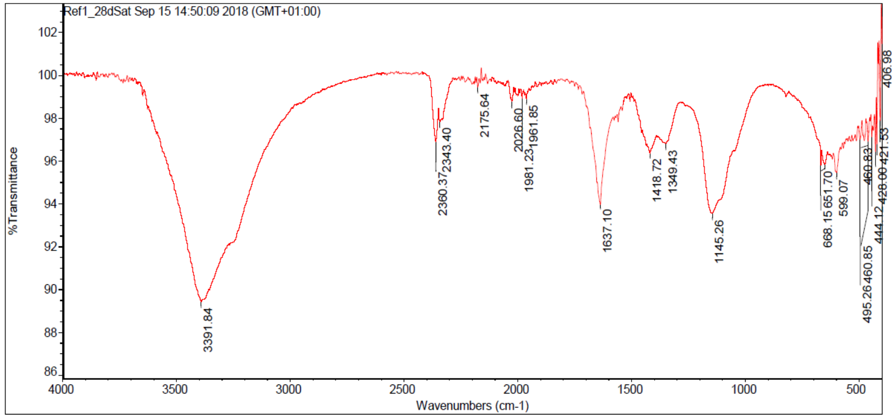

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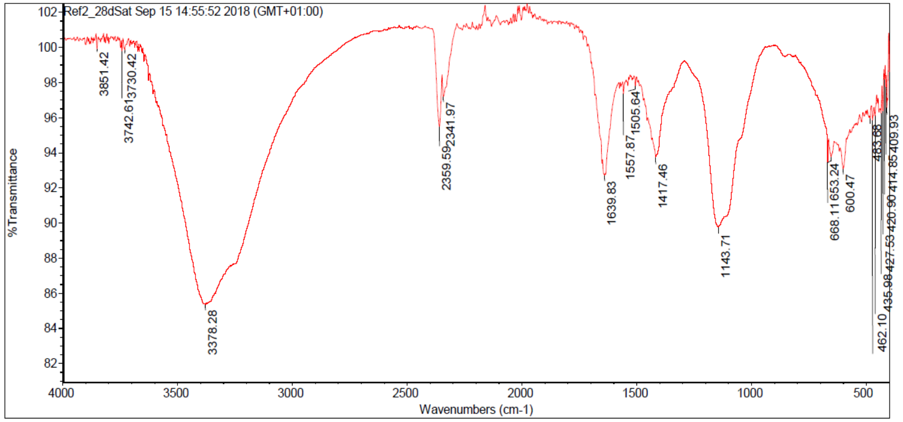

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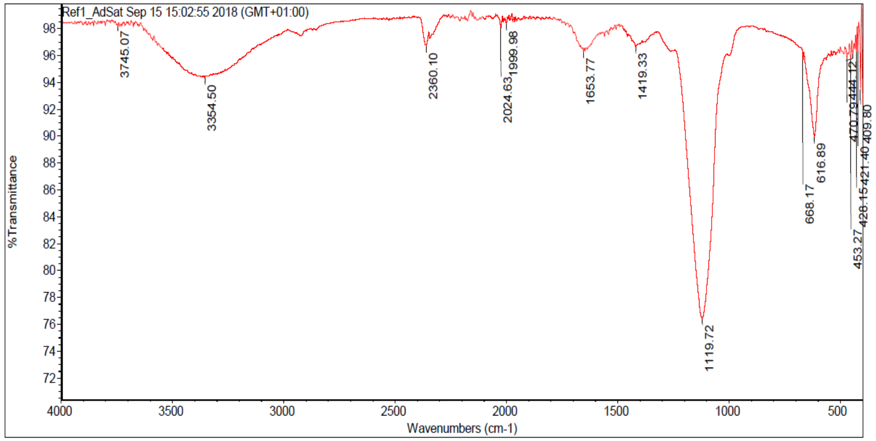

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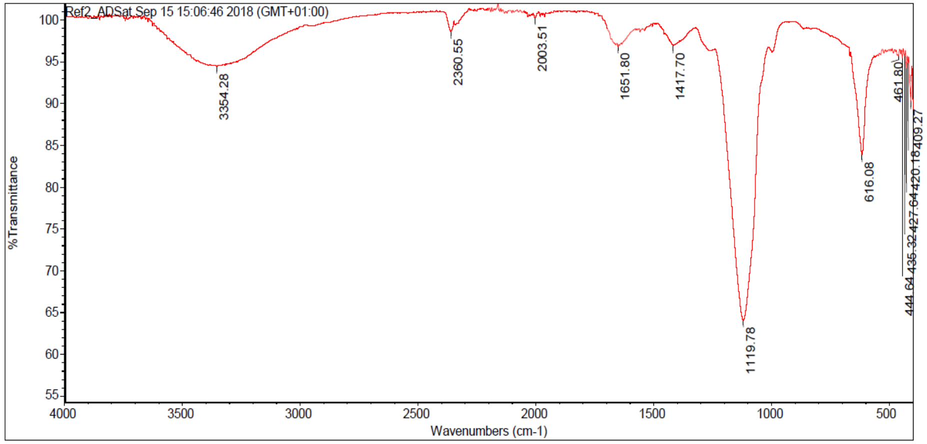

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