Characterizing the Removal of Antibiotics in Algal Wastewater Treatment Ponds: A Case Study on Tetracycline in HRAPs

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Abstract

Antibiotics are ubiquitous pollutants in wastewater, owing to their usefulness in both animal and human treatment. Antibiotic pollution is a growing concern because of the risk of encouraging antibiotic resistance in wastewater treatment (WWT) systems and downstream of effluent discharge. The aim of this thesis was to investigate the fate of antibiotics in algal WWT ponds, which have unique ecological and environmental characteristics (e.g. presence of algae; diurnal variation in pH, dissolved oxygen, and temperature) compared with conventional biological WWT.

The research in this thesis focused on a case study of the fate of tetracycline (TET, an antibiotic) in high rate algal ponds (HRAP). Indoor lab scale HRAP studies were used to investigate the fate of TET under several operating conditions. Outdoor pilot scale studies (900 L and 180 L HRAPs) under Oceanic and Mediterranean climates were used to validate the lab scale findings. Results showed that high removal (85% to >98%) of TET was possible in the lab and pilot scale HRAPs with HRTs of 4 and 7 days. Sorption was consistently a low contributor (3-10% removal by sorption) during continuous HRAP studies, based on the amount of TET extracted from biomass. Batch experimentation was used to further distinguish mechanisms of TET removal. The majority of TET removal was caused by photodegradation. Indirect photodegradation of TET was dominant over direct photolysis, with 3-7 times higher photodegradation observed in wastewater effluent than for photodegradation in purified water during batch tests incubated in sunlight. Under dark conditions sorption was the dominant removal mechanism, and biodegradation was negligible in batch tests since aqueous TET removed was recovered (± 10%) by extraction of sorbed TET from the biomass.

Irreversible abiotic hydrolysis was not observed during TET removal batch tests in purified (MQ) water.

A kinetic model was developed and used to predict TET removal in the pilot HRAPs, based on parameters derived from batch experiments. The model predictions for aqueous TET concentrations were successfully validated against initial TET pulse tests in the 180 L pilot scale HRAP. However TET removal decreased in subsequent pulse tests in the pilot HRAP, resulting in over-prediction of TET removal by the kinetic model. This decrease in TET removal was associated with decrease in pH, dissolved oxygen concentrations, and biomass settleability, but causal relationships between TET removal and these variables could not be quantified. Until the predictive kinetic model is developed further, this model may serve as a preliminary estimate of TET fate in algal WWT ponds of different design and operation. Future research should also investigate the potential formation and toxicity (including antibiotic efficiency) of TET degradation products, but this was outside the scope of this thesis. Predictions from the model were sensitive to the daily light intensity, suggesting that TET removal would be reduced in the winter months.

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Table of Acronyms

Acronym	Full Name
BOD	Biological Oxygen Demand
CMP	Chloramphenicol (antibiotic)
COD	Chemical Oxygen Demand
CPX	Ciprofloxacin (antibiotic)
HPLC	High Performance Liquid Chromatography
HRAP	High Rate Algal Pond
HRT	Hydraulic Retention Time
IC	Inorganic Carbon
MQ (water)	Milli-Q grade purified water
PAR	Photosynthetically Active Radiation (400-700 nm)
PNCC-WWTP	Palmerston North City Council Totara Rd Wastewater Treatment Plant
RO (water)	Reverse Osmosis grade purified water
SCC mix	Mixture of antibiotics: Sulfanilamide, Ciprofloxacin, and Chloramphenicol
SFL	Sulfanilamide (antibiotic)
SMX	Sulfamethoxazole (antibiotic)
SPE	Solid Phase Extraction
SRT	Solids Retention Time (a.k.a. Sludge Retention Time)
STS mix	Mixture of antibiotics: Sulfanilamide, Tetracycline, and Sulfamethoxazole
TET	Tetracycline (antibiotic)
TN	Total Nitrogen

TOC	Total Organic Carbon
TSS	Total Suspended Solids
UV	Ultraviolet
UVA	Ultraviolet light (320-400 nm)
UVB	Ultraviolet light (290-320 nm)
VSS	Volatile Suspended Solids
WSP	Waste Stabilisation Pond
WW	Wastewater
WWT	Wastewater treatment

1. Introduction

1.1 Background

The presence of antibiotics in wastewater (WW) is ubiquitous, due to their usage in both human and animal treatment and the subsequent excretion of antibiotics in the faeces and urine. Wastewater treatment (WWT) is therefore a major gateway for the release of antibiotics to the environment. Antibiotics have been shown to encourage resistant bacterial populations at concentrations 10-100 times lower than the minimum inhibitory concentrations. This release of antibiotics via WWT is therefore concerning as a possible contributing factor to the increasing risk of antibiotic resistant infections, a major human health concern.

Algal WWT ponds (or lagoons) are low-cost WWT operations that are commonly used worldwide for low-population settings (e.g. rural towns, farms). The shallow ponds are designed to support algal growth, which provides oxygen for the biological treatment of the WW. The integration of intensive algal WWT ponds such as the High Rate Algal Pond (HRAP) with municipal WWT systems has been suggested in order to utilise the high biomass productivity as a method for energy production and nutrient recovery.

1.2 The problem and aim

The design of algal WWT ponds results in the establishment of unique environmental conditions (e.g. light, pH, and oxygen levels) in comparison with other biological WWT. The literature (reviewed in Section 2) suggests that these unique conditions could affect the degradation and removal of antibiotics, especially by allowing photodegradation mechanisms to contribute to antibiotic removal, but the direct (biodegradation/sorption) or indirect (e.g. pH changes) effects of algal growth on the

fate of antibiotics in these systems is unclear. Yet there is currently a scarcity of information regarding the fate of antibiotics in algal WWT ponds in order to assess the risks regarding the release of antibiotics to the environment through algal WWT ponds (see Section 2). Knowing that antibiotic release can encourage antibiotic resistance, the following questions were raised: 1) Do algal WWT ponds remove antibiotics well? 2) How are antibiotics removed - what are the dominant removal mechanisms? 3) Can we predict antibiotic removal in algal WWT ponds?

The aim of this thesis was therefore to study the fate of antibiotics in algal WWT ponds, especially how diurnal changes in sunlight exposure affects the antibiotic removal mechanisms within algal WWT ponds.

As this subject was too large to consider every antibiotic and type of algal pond, and in the absence of research already conducted on the subject, a case study on tetracycline (TET) in HRAP was conducted as the primary focus – this choice is discussed in Section 3.2.1.

1.3 Research hypothesis and objectives

Based on the literature review (Section 2) the following research hypotheses were proposed at the beginning of this research:

The fate of antibiotics in algal WWT will be affected by photo-based degradation pathways and the diurnal variation in pH and DO caused by photosynthetic activity. The characteristics of sorption and biodegradation in algal ponds will be significantly different than during conventional biological treatment due to the presence of photosynthetic microorganisms. The understanding of the fate of antibiotics during algal WWT will allow optimisation of algal WWT processes for the removal of antibiotics.

The four objectives for this thesis are listed below, with specific research questions listed under each objective:

1. To determine how efficiently TET is removed from WW in HRAP systems, and how sensitive TET removal is to changes in scale, operation, and environmental conditions.
    - Is TET-containing WW passing through HRAP systems likely to pose a high risk to environments downstream, or is TET sufficiently well-removed in HRAPs that the risk of TET affecting environments downstream is low?
2. To determine the relative significance of potential TET removal mechanisms such as photodegradation, biodegradation, sorption, and hydrolysis.
    - Does algae activity affect TET removal directly via biodegradation or sorption onto the biomass, or do abiotic degradation processes such as photodegradation and hydrolysis dominate TET removal?
3. To identify any potential links between TET removal rates in the presence of algal-bacterial biomass and the monitored environmental and WW conditions.
    - Does algae activity affect pollutant removal indirectly via pH and dissolved oxygen concentration fluctuations?
    - If TET removal kinetic rates depend on temperature changes or sunlight intensity (e.g. for photodegradation), how are seasonal changes likely to affect TET removal in algal WWT ponds?
4. To predict potential TET removal in a variety of algal pond designs.
    - Is TET removal sensitive to design differences in a variety of algal ponds, or is the overall removal similar?

2. Literature Review

This literature review introduces the reasons that antibiotic release into the environment presents an environmental and human health risk, introduces the design, operation, and environmental conditions of algal WWT, and reviews current knowledge of antibiotics in other WWT and environmental settings. The conclusion of this literature review suggests the potential fate of antibiotics in algal WWT ponds based on this knowledge and provides objectives for the research in this thesis.

This literature review formed the basis for an article published by Journal of Hazardous Materials (Norvill et al., 2016). The main difference between the published literature review and the one presented in this section is that the published literature review had an expanded scope, including more emerging contaminants than antibiotics alone.

2.1 Introduction

There is an increasing awareness of the human health and ecological risks caused by the widespread environmental occurrence of emerging contaminants such as pharmaceuticals, personal care products, nanomaterials, and perfluorinated compounds (Arnold et al., 2013; Brookes et al., 2014; Lapworth et al., 2012; Marcoux et al., 2013). Among these substances, antibiotics are of particular concern for three main reasons: i) antibiotics are widely used in human and veterinary medicines (González-Zorn and Escudero, 2012; Kemper, 2008; Lupo et al., 2012; van den Bogaard and Stobberingh, 1999); ii) antibiotics can impact ecological functions and microbial community structure (Ding and He, 2010); iii) antibiotics can encourage the spread of antibiotic resistance at sub-therapeutic concentrations (Aminov, 2009; Gullberg et al., 2011). The encouragement of antibiotic resistance is particularly concerning, as resistant infections cause health and societal costs in the ten billion dollars annually in the US, and deaths in the ten thousands annually in Europe alone (Bush et al., 2011). Though the management of antibiotic usage may become stricter, these compounds cannot be prohibited because they save lives (Pruden et al., 2013; Teixeira Rodrigues et al., 2013), meaning their presence in WW will continue to be ubiquitous.

Because 60 - 90% of the antibiotic dose delivered is excreted via faeces and urine, WWT is a major release point of antibiotics into the environment (Christensen et al., 2006; Hirsch et al., 1999; Sarmah et al., 2006). Typical concentrations in waters downstream of WW discharge range from 0.1-2.0 µg L^{-1} (Haggard et al., 2006; Hirsch et al., 1999; Sarmah et al., 2006; Zheng et al., 2011) but surface water concentration in high intensity agricultural areas can reach 40-400µg L^{-1} (Pei et al., 2006). Antibiotic concentrations in manure can reach from 1 µg kg^{-1} up to 5000 mg kg^{-1} in manure

slurries, and these slurries can be treated via WWT methods or solid waste processing prior to field application (Chee-Sanford et al., 2009; Selvam et al., 2012). However, this literature review focuses on WWT, rather than treatment of solid waste treatment processes (e.g. composting: Selvam et al., 2012).

Because wastewater discharge is a major source of antibiotic entry into the environment over other sources (e.g. runoff), WWT provides a focal point for targeting antibiotic removal. Although the removal of antibiotics during conventional and advanced WWT has been intensively researched (Kümmerer, 2009a; Michael et al., 2013; Monteiro and Boxall, 2010a; Sim et al., 2011), little is known on the fate of these contaminants in algal WWT ponds [Box 1]. This lack of knowledge is of concern because thousands of communities worldwide depend on algal pond systems for WWT (Archer and Mara, 2003; Heaven et al., 2003; Mara and Pearson, 1998; U.S. EPA, 2011). Thus, despite the societal, economical and environmental relevance of these technologies, only three laboratory scale studies have hitherto specifically investigated the fate of antibiotics during algal WWT (de Godos et al., 2012; Dzomba et al., 2015; Guo and Chen, 2015), and one recent fate study on pharmaceuticals in wetlands and ponds that included 3 antibiotics (Rühmland et al., 2015) . Despite the paucity of directly relevant data, considerable insights can be gained from studies of the removal of antibiotics in conventional systems (e.g. activated sludge: Michael et al., 2013; Monteiro and Boxall, 2010), and studies focusing on removal mechanisms potentially relevant in algal WWT ponds – namely photodegradation in rivers and lakes (López-Peñalver et al., 2010; Prabhakaran et al., 2009), and biodegradation and biosorption of similar pollutants by algae and algae-bacterial consortia (Hom-Diaz et al., 2015; Matamoros et al., 2015a, 2009; Muñoz and Guieysse, 2006). This literature review analyses this multi-disciplinary knowledge in light of the unique and highly variable ecological and

environmental conditions occurring in algal WWT ponds in order to identify the most likely routes of antibiotic removal in these systems and thus identify critical research needs in the area. Knowledge on the fate of other emerging contaminants (e.g. estrogens) is discussed when deemed particularly relevant to explain antibiotic removal. Because of the broad range of scientific disciplines concerned (from applied phycology to environmental engineering), Section 2.2 of the literature review summarizes current relevant knowledge on algal WWT. Based on this knowledge, Sections 2.3-2.6 systematically discusses the relevance of current knowledge on antibiotics sorption, biodegradation, photodegradation, and hydrolysis during conventional WWT and surface waters with regards to the specific ecological and environmental conditions occurring in algal WWT ponds. Section 2.7 then discusses risks related to antibiotic degradation products and antibiotic resistance in algal WWT ponds and WWT. Section 2.8 concludes this review and provides objectives for the experimental research.

Box One: Definitions

In this thesis, the terms ‘algal’, ‘conventional’, and ‘advanced’ describing wastewater treatment (WWT) systems are defined as:

- Conventional WWT: biological treatment processes involving activated sludge.
- Advanced WWT: processes which require addition of large amounts of energy (other than aeration and pumping), or consume resources such as adsorbents or chemicals. Examples included advanced oxidation processes, disinfection, and activated carbon filters
- Algal WWT ponds: biological treatment processes designed to encourage photosynthetic growth, excluding macrophyte systems such as wetlands. Although the term ‘algal WWT ponds’ is used for simplicity, the phototrophic biology includes both cyanobacteria and algae. Examples include HRAP, facultative ponds, and maturation ponds.

2.2 Algal-based wastewater treatment

Algal WWT ponds are low-energy inputs systems relying on long hydraulic retention times for high removal efficiencies. The three main types of algal WWT ponds used for WWT include facultative ponds, maturation ponds, and HRAP. Facultative and maturation ponds are often collectively referred to as waste stabilization ponds (WSP) or WWT lagoons (Crites et al., 1998; FAO, 1992; Shilton and Walmsley, 2005). The term 'oxidation pond' may also be used and 'high rate oxidation pond' (HROP) has been used as an alternative to HRAP (Azov and Shelef, 1982). However, the term 'oxidation pond' may also refer to mechanically aerated ponds with low algae concentration. Facultative and maturation ponds are used all around the world for WWT in provincial areas (Archer and Mara, 2003; Heaven et al., 2003; Mara and Pearson, 1998).

Facultative ponds are usually 1-2 m deep and receive either raw WW or effluent from an anaerobic waste treatment pond. Maturation ponds are 1-1.5 m deep and are designed for pathogen removal: These systems receive effluents from facultative ponds or conventional WWT systems after most of the organic load has been removed. HRAPs are 0.2-0.5 m deep and the pond water is circulated around a 'raceway' path by paddle wheel. HRAPs may receive effluents from a previous waste treatment pond, effluents following screening or settling processes, or even raw WW if the presence of debris, fat, and suspended solids does not significantly impact their performance (i.e. suspended solids may reduce light penetration or settle in the pond).

There are several key operational and design differences between algal WWT and other biological systems, as shown in Table 1. Algal WWT ponds have very high surface area to volume ratios (and therefore high light exposure) and are operated under long hydraulic retention times (HRTs). This design and operation encourages phototrophic growth which provides oxygen for aerobic biodegradation. Photosynthesis consumes CO_2 and releases oxygen, resulting in supersaturated dissolved oxygen (DO) levels during the day (130-300% saturation possible: Kayombo et al. 2000; Muñoz & Guieysse 2006). The CO_2 consumption also shifts the carbonate balance, which can raise the pH up to 8.5 - 11 during the day (Kayombo et al., 2000; Muñoz and Guieysse, 2006). Diurnal (daily) variations in pH and DO can therefore be very significant in algal WWT ponds due to the change in sunlight intensity and the associated photosynthetic production (El Ouarghi et al., 2003; García et al., 2006; Picot et al., 2005a). The high surface to volume ratio of algal WWT ponds also results in large daily fluctuations in temperature, especially in HRAP, due to heating from sunlight and large surfaces for convective and evaporative heat exchange (Béchet et al., 2011). Seasonal variations of temperature and light intensity also have a major impact on environmental conditions and pond performance (Mara and Pearson, 1998).

The ecology of algal WWT ponds is characterized by a high phototrophic activity [Table 1]. For simplicity, ‘algae’ and ‘algal’ will be henceforth used as broad terms including both cyanobacteria and true algae, unless otherwise stated. Likewise, the term ‘bacterial’ will be used to describe any non-phototrophic microorganism in the system and mainly refers to heterotrophs contributing the organic pollutant biodegradation in the context of this thesis. Because the design and environmental conditions affect the growth of algal species, the ratios of algal and bacterial biomass and dominance of

different species vary seasonally (García et al., 2000; Oron et al., 1979). The type of relationship between the algae and bacteria depends strongly on the limiting factor for growth. Algae can produce oxygen and organic acids for bacteria, and bacteria can produce CO_2 and vitamins for algae (Croft et al., 2005; Liu et al., 2012a; Nishio et al., 2013). Competition for nutrients such as phosphorous may cause adversarial interactions (Liu et al., 2012a), possibly including production of natural antibiotics by algae (Cole, 1982).

Table 2 summarises how the specific design and environmental conditions unique to algal WWT ponds (high light exposure, diurnal pH and DO variations, long HRT, ecology, etc.) may impact the removal of antibiotics. Long term associations and interactions between algae and bacteria in biofilms and flocs have been shown to increase resource availability for algae and bacteria (Peterson et al., 2011), which can influence biodegradation. Sorption may be affected by the different properties of algal/bacterial biomass (Jelic et al., 2012). Although both total biomass concentrations and bacterial cell counts are lower in algal WWT ponds than in activated sludge type systems, biological performance is also related to retention time and overall biomass productivity. Hence, the negative impact of the comparatively low cell concentration occurring during algal WWT may be offset by the high volume (i.e. the long HRT; Table 1) and high biomass productivity due to photosynthetic carbon fixation. Also, the sediment layer in algal WWT ponds has both a large surface area and high microbe concentrations. The potential influence of these unique conditions on antibiotic removal is discussed further in the following sections.

Table 1: Comparison of typical WWT processes (some of these processes can be combined during WWT).

Parameter	Facultative Pond	Maturation Pond	High Rate Algal Pond (HRAP)	Activated Sludge	Anaerobic UASB[a]
Depth	1-2 m	1-1.5 m	0.1-0.5 m	2.4-6 m	3-10 m
Hydraulic Retention Time (HRT)	20-50 days	3-15 days	1-14 days	3-8 hours	6-14 hours
Sludge/Solids Retention Time (SRT)	20-50[b] days	3-15[b] days	1-28 days	3-15 days	NA
Biomass conc. (MLVSS[c])	0.04-0.16 g/L	0.03-0.07 g/L	0.1-0.3 g/L	1.2-4.0 g/L	6-13 g/L
Typical microbiology type present[d]	Phototrophs; Aerobic chemotrophs; Anaerobic chemotrophs	Phototrophs; Aerobic chemotrophs	Phototrophs; Aerobic chemotrophs	Aerobic chemotrophs	Anaerobic chemotrophs
pH	6.5-8.5 with diurnal fluctuation	6.5-10 with diurnal fluctuation	controlled at 7-8, or 7-11 with diurnal fluctuation	6-9 (generally kept near 7)	6.8-7.5
Dissolved Oxygen	Surface[e] (daytime): aerobic to supersaturated (>13 mg/L DO possible). Sludge layer: anaerobic. Pond anoxic at night	Fully aerobic to supersaturated during daytime (>15 mg/L DO possible). Pond may be anoxic at night.	Aerobic to supersaturated during daytime (>30 mg/L possible). Pond may be anoxic at night, if heavily loaded.	0.5 - 4 mg/L	0 mg/L
BOD loading	8-20 g m^{-2} d^{-1} (0.008-0.02 kg m^{-3} d^{-1}, for a 1 m deep pond)	NA (not designed for BOD removal)	Up to 35 g m^{-2} d^{-1} (Up to 0.12 kg m^{-3} d^{-1} for a 0.3 m deep pond)	0.3-1.6 kg m^{-3} d^{-1}	2-8 kg m^{-3} d^{-1}

Mixing/ Aeration	Inlet momentum; Wind; Sometimes a small mechanical aerator	Inlet momentum; Wind	Paddle wheel; May include CO_2 addition	Intensive aeration with mixing	Inlet momentum
References	Kayombo et al. (2000) Mara and Pearson (1998), Mara et al. (1992), García et al. (2000), Picot et al. (2005)	Craggs et al. (2003), Mara and Pearson (1998), Mara et al. (1992), Archer and Mara (2003)	Craggs et al. (2003), Muñoz and Guieysse (2006), El Hamouri et al. (2009), Park et al. (2013), Posadas et al. (2015)	Metcalf & Eddy (2003), Eckenfelder (1998)	Metcalf & Eddy (2003), Yu et al. (2001)

[a] Upflow Anaerobic Sludge Bed: Since there are various anaerobic systems with many different characteristics, one example was chosen for comparison

[b] There is generally no recirculation of solids for facultative ponds and maturation ponds, so it can be considered that SRT=HRT. However, the sludge that settles to the bottom is rarely removed, so the SRT for some solids/biomass may be on the order of years in the sludge layer.

[c] MLVSS= Mixed Liquor Volatile Suspended Solids: organic fraction of suspended solids commonly used as a measure of biomass concentration. Values for algal WWT ponds taken from TSS (Total Suspended Solids) given in literature: the organic fraction is very high so MLVSS≈TSS.

[d] Phototrophs use light as an energy source, chemotrophs use organic carbon as an energy source

[e] Depending on mixing and loading, the bulk of the facultative pond is typically aerobic near the band algal of growth (within the top 0.5 m of water), with DO decreasing to anoxic levels deeper in the pond as bacteria consume the oxygen

Table 2: Potential effects of key process conditions upon antibiotic removal during algal and conventional activated sludge WWT.

Parameter	Impact on antibiotic removal	Algal WWT processes (Typical)	Activated Sludge (Typical)
Mixing	Hydraulic short-circuiting reduces retention time for a fraction of pollutants, reducing overall efficiency for first order reactions.	Ponds have low mixing intensity; they are mainly mixed by wind, convection, and inlet flow. Facultative ponds can be stratified sometimes. In HRAPs, paddle wheels increase mixing.	Well-mixed due to aeration.
Hydraulic Retention Time (HRT)	HRT influences the time available for degradation and removal to occur.	High HRT (days to weeks)	Low HRT (hours)
Biomass concentrations	The significance of biodegradation and sorption is typically correlated to biomass concentration (Dorival-García et al., 2013).	Algal WWT ponds have low biomass concentrations (0.03-0.3 g/L, Table 1) and bacterial cell counts, particularly in maturation ponds (Davies-Colley, 2005).	High biomass concentrations (1.2-4 g/L) (Metcalf & Eddy 2003)
Biomass productivity	High productivity increases organic biomass which may then act as a sorbent.	High biomass productivity due to photosynthesis in addition to heterotrophic growth.	High heterotrophic growth (net productivity depends on SRT).
Microbiology	Biodegradation kinetics and sorption characteristics change with microbial properties(Batt et al., 2007; Jelic et al., 2012).	Both algae and aerobic bacteria are present. Anaerobic bacteria are also found in facultative ponds.	Aerobic and facultative bacteria
Light exposure	Light exposure enables photodegradation	High light exposure	Minimal light exposure
Temperature	Temperatures affect kinetic rates, with higher temperatures typically increasing kinetics	High surface area to volume ratios means algal WWT ponds are significantly affected by climatic conditions and this can cause large daily and seasonal temperature variations.	Temperatures will be affected seasonally, but daily variations are minor due to short HRTs and compact nature
pH	pH affects antibiotic structure, sorption, and removal kinetics. Extreme pH can inhibit microbes. (Monteiro and Boxall, 2010b; Muñoz and Guieysse, 2006).	pH fluctuates diurnally due to daytime photosynthetic activity consuming CO_2 faster than bacteria and diffusion replenish it (Pearson, 2005).	Activated sludge is usually operated around neutral pH with small variations (Metcalf & Eddy 2003).
Dissolved Oxygen	DO may influence microbiology (and therefore biodegradation and biosorption). DO may also affect photo-oxidation mechanisms (Glover and Rosario-Ortiz, 2013).	Large daily variations can be observed from super-saturation to negligible oxygen present. DO may also vary with depth, especially during stratification in facultative ponds (Pearson, 2005).	DO levels are maintained around 2 mg/L (≈20% saturation) (Metcalf & Eddy 2003).

2.3 Sorption

2.3.1 Current knowledge of antibiotic sorption in conventional systems

The sorption of antibiotics to the solids found and generated during WWT is highly dependent on the structure of the compound, the properties of the solids, and environmental conditions. Regarding antibiotic sorption to activated sludge, sulphonamides, tylosin, and aminoglycosides are generally weakly sorbed whereas quinolones and tetracyclines have high sorption affinities (Batt et al., 2007; Dorival-García et al., 2013; Prado et al., 2009; Zhou et al., 2013c).

Various isotherm models (e.g. Langmuir, Freundlich) are traditionally used to quantify the partitioning properties of chemicals between solid and liquid phases. For trace contaminants like antibiotics, a simple partition ratio (K_d, Table 3) is often used for this purpose because the solid phase is usually not saturated (this assumption should always be verified in case the solids have a limited number of adsorption sites; Limousin et al. 2007).

Because sorption isotherms measure an interaction between the antibiotic and the solids, the sorption coefficients depend on the structure of the antibiotic and the properties of the solids, as further discussed below. In addition, temperature, pH, and the ionic background of the liquid phase can affect sorption coefficients (Limousin et al., 2007). Increasing temperature usually reduces sorption because sorbed pollutants are in a lower energy state than dissolved pollutants. The effect of temperature on the sorption of antibiotics is exemplified in Table 3, with a 4-fold increase in the K_d measured when temperature was reduced from 28°C to 9°C (Dorival-García et al., 2013). This temperature range is relevant to expected values for WWT pond systems.

Because the ionic background affects sorption by altering charge interactions between antibiotics and solids, regional variations in water hardness (Briggs and Ficke, 1977) may cause variations in antibiotic sorption. Water hardness may also increase apparent sorption due to precipitation of antibiotic-ion complexes, for example calcium and other cations can cause precipitation of tetracyclines (Daughton and Ternes, 1999). The charge interactions between antibiotics and solids also depend highly on the pH (Li and Zhang, 2010; Zhou et al., 2013c), which changes the antibiotics and solids structurally. This is illustrated in Table 3, as ciprofloxacin sorption to either aerobic or anaerobic sludge was shown to be over twice as high at neutral pH than under acidic or basic pH ranges (Zhou et al., 2013c). Antibiotics with different structures will have different optimal pH ranges for sorption to occur. Little is known on the impact of pH on the sorption of antibiotics onto biosolids generated during conventional secondary WWT, probably because these processes typically operate at neutral and steady pH. In contrast, the effects of pH on antibiotic sorption to soils is well documented but the extrapolation of these findings to biosolids is risky because soils have a higher mineral content and a lower lipid content than sewage sludge (Kümmerer, 2009b; Thiele-Bruhn, 2003). The data on soils may be useful for WWT systems with clay liners; however that is not a focus in this thesis.

Table 3: Examples of measured sorption partition coefficients [K_d] varying with environmental conditions, antibiotic structure, and solid type

Parameter	Parameter Levels	K_d [L/kg]	Antibiotic	Conditions	Reference
Temperature	9°C	2806 1194 - 3746	Ciprofloxacin Range for 6 quinolones	Sludge from pilot-scale MBR, with HRT=0.5 days, SRT=32 days, fed with urban WW $C_{antibiotic}$=0.5mg L^{-1} MLSS=13 g L^{-1}	Dorival-Garcia et al. (2013)
	28°C	690 458 - 1270	Ciprofloxacin Range for 6 quinolones		
	38°C	522 240 - 872	Ciprofloxacin Range for 6 quinolones		
Solid type	Influent SS [a]. (SS = 0.089 g L^{-1})	405 ± 109 132 ± 20	Azithromycin Clarithromycin	Solids/sludge from different stages in a municipal integrated WWT plant Concentrations of antibiotic in the WWT system 50-80 and 100-200 ng L^{-1} respectively for AZT and CRT	Jelic et al. (2012)
	Thickened Sludge [b]. (SS = 36.4 g L^{-1})	444 ± 133 461 ± 36	Azithromycin Clarithromycin		
	Digested Sludge [c]. (SS = 24.7 g L^{-1})	367 ± 89 715 ± 261	Azithromycin Clarithromycin		
pH	pH 4 (aerobic) pH 6-8 (aerobic) pH 10 (aerobic) pH 4 (anaerobic) pH 6-8 (anaerobic) pH 10 (anaerobic)	80 300-360 80 407 1000 - 1330 45	Ciprofloxacin	Partition coefficients not given directly, values calculated from the adsorption ratio and experimental conditions: 0.1 mg L^{-1} initial ciprofloxacin, 3.0 g L^{-1} MLVSS inhibited aerobic or anaerobic sludge	Zhou et al. (2013c)

[a] Influent SS: Suspended solids from municipal WWT plant.
[b] Thickened Sludge: Sludge from Preanoxic/Anaerobic/Anoxic/Oxic series treatment, mixed with the organic fraction of municipal solid wastes and thickened for anaerobic digestion.
[c] Digested sludge: Mechanically dewatered sludge exiting the anaerobic digestion process (fed with the above thickened sludge).

The concentration and composition of the biological sludge generated during WWT is influenced by design and operational parameters such as the hydraulic retention time (HRT) and the sludge retention time (SRT), which thus affect pollutant sorption. For example, Kim et al. (2005) showed that reducing the SRT reduced tetracycline removal efficiency in an activated sludge sequential batch reactor whereas the HRT did not affect removal efficiency despite changes in biomass concentrations (biodegradation was not observed during these experiments). The impact of solid composition upon antibiotics sorption is evidenced in Table 3 as the partition coefficient of clarithromycin onto the suspended solids found in influent WW was reported to be 5-fold lower than the partition coefficient onto the biological sludge from an anaerobic digester.

It is important to consider that sorption only provides temporary risk reduction. On the positive side, sorption to cellular sites may be a preliminary step to biodegradation (Esparza-Soto and Westerhoff, 2003). Negatively, antibiotics may subsequently desorb and return to the liquid phase or leach out during sludge treatment and/or disposal (Song et al., 2010). The end use or disposal of the sludge should therefore be carefully considered.

2.3.2 Potential of antibiotic sorption in algal WWT ponds

As explained in Section 2.3.1, the significance of antibiotic sorption during WWT is highly specific to the antibiotic structure (e.g. hydrophobicity and functional groups binding onto the sorbent), the physical and chemical properties of the sorbent (e.g. hydrophobicity, surface chemistry, and surface area of solids), and environmental conditions affecting sorption thermodynamics (e.g. pH) and kinetics (e.g. temperature). The specific composition of biomass in algal WWT may therefore change sorption

characteristics. Matamoros et al. (2015) reported that only hydrophobic pharmaceuticals were significantly sorbed onto HRAP biomass, which agrees with the findings from studies conducted on biosolids from activated sludge discussed in Section 3.1. While rapid (3 hr) sorption of estrogenic hormones onto biomass was reported during algal WWT (Shi et al., 2010), these findings may be tentatively extrapolated only to hydrophobic antibiotics as no study has specifically quantified antibiotic sorption onto biomass containing algae as and many antibiotics are relatively hydrophilic (Monteiro and Boxall, 2010b). The fluctuating pH in algal WWT ponds may be especially important to sorption processes, affecting both the antibiotic structure and the structure of the sorbents present. Extracellular polymeric substances (EPS), a component of flocs and biofilms in both bacterial WWT (esp. activated sludge) and algal WWT ponds, are also known to change in composition (e.g. carbon content, abundance of chlorophyll/cells/EPS) depending on external factors such as light intensity and presence of biofilm grazers (Barranguet et al., 2005). The amount and composition of EPS in algal WWT ponds may therefore affect the significance of antibiotic sorption in algal WWT ponds compared with conventional WWT systems.

2.4 Biodegradation

2.4.1 Current knowledge of antibiotic biodegradation in conventional systems

Many antibiotics are not 'readily biodegradable' when supplied at mg L^{-1} initial concentrations (Alexy et al., 2004) based on OECD readily biodegradability guidelines (60% mineralisation within 10 days of 10% mineralisation). This lack of 'readily biodegradability' does not exclude biodegradation during wastewater biological treatment (conventional or algal based) because the standard tests do not account for the potential impact of lower antibiotic concentrations, co-metabolism, and microbial acclimation—a mechanism constantly occurring during continuous treatment with diverse microbial communities (Alexy et al., 2004; Guieysse and Norvill, 2014). Biodegradation involves specific enzymes that catalyse the transformation of hazardous pollutants. For biodegradation to take place in a specific ecosystem, the microbial populations exposed must therefore both possess and express the genetic abilities required (Guieysse and Wuertz, 2012). In diverse ecosystems such as those found in algal WWT ponds, the genetic abilities required to degrade biomolecules with relatively simple and common structures, such as most antibiotics, are generally available. The required gene expression and/or enzyme activity can however be repressed due to the low concentrations involved because there is no 'incentive' for cells to expend energy for carrying out the targeted reaction(s), e.g. no need to reduce toxicity or use the pollutants as carbon and/or energy sources given the availability of other food sources. Fortunately, pollutant degradation can also occur via the action of non-specific enzymes synthesized for another primary purpose.

For biodegradation to be quantitatively significant during WWT, the rates of biodegradation must be significant with regards to treatment times (e.g HRT),

competent microbial population must be present and active, environmental conditions must be suitable (e.g. suitable temperature, availability of co-reactants), pollutant must be available, and competitive removal mechanisms must remain limited (e.g. sorption and photodegradation of target pollutant, or competitive biodegradation of other compounds). For example, while many antibiotics are relatively small hydrophilic and bioavailable compounds (Michael et al., 2013), the medium to high sorption affinities of some antibiotics may prevent their biodegradation (Li and Zhang, 2010). Several studies have shown that antibiotic biodegradation is influenced by antibiotic structure and environmental conditions: the biodegradation of the antibiotics listed in Table 4 varies between 0.1 - 9 L day^{-1} $g^{-1}_{biomass}$ under aerobic conditions, depending on the antibiotic tested. If the rates of antibiotic biodegradation are inherently low during WWT due to the very low antibiotic concentration involved, biodegradation may be improved by enabling the development of slow-growing microbial populations through WWT operation with long sludge retention times (SRT) (Batt et al., 2007). Long sludge retention times can be achieved by either using large reactor volumes (long HRTs) or by recirculating/retaining the biomass in a small reactor (long SRTs with short HRTs). The use of membrane bioreactors (MBRs) allows operation at very high solids/biomass concentrations, and indeed appears to improve elimination of many antibiotics compared to conventional activated sludge (Kovalova et al., 2012; Larcher and Yargeau, 2012; Michael et al., 2013), although sorption to the membrane or sludge may also contribute to the increased removal of antibiotics.

Table 4: Comparison of biodegradation rate variability of selected antibiotics

Antibiotic	Rate constant normalised for biomass concentration [L day^{-1} $g^{-1}_{biomass}$]	Description of system	Reference
Erythromycin	6 (aerobic) 0.2 (anoxic)	2 L reactors were inoculated from a conventional activated sludge pilot plant, operated aerobically (recirculation ratio 40) or anoxically (recirculation ratio 30), each fed with synthetic WW with an HRT of one day.	Suarez et al. (2010)
Roxithromycin	9 (aerobic) 0.15 (anoxic)		
Sulfamethoxazole	0.3 (aerobic) n.a. (anoxic)		
Trimethoprim	0.15 (aerobic) n.a. (anoxic)		
Cefalexin	2.82	Activated sludge batch tests were conducted with 1 L volumes, differentiating biodegradation by parallel tests with inhibited activated sludge (2.21g/L).	Li and Zhang (2010)
Sulfamethoxazole	0.12		
Sulfadiazine	0.17		

2.4.2 Potential for antibiotic biodegradation in algal WWT ponds

Various studies have demonstrated the ability of algae, and algae-bacteria communities, to biodegrade organic pollutants such as hormones, oils, organic solvents, and phenols at high concentrations in industrial wastes (Muñoz and Guieysse, 2006; Peng et al., 2014; Subashchandrabose et al., 2011). Hom-Diaz et al. (2015) reported that algal biodegradation accounted for 42-54% of the observed removal of two estradiols (hormones) during the treatment of an anaerobic digester centrate. In domestic WW, Matamoros et al. (2015) reported good removal of trace concentrations (µg L^{-1}) of pharmaceuticals in a pilot HRAP and attributed the removal to photodegradation and biodegradation for most compounds. Sorption may precede the biodegradation of hydrophobic pollutants, as shown by Shi et al. (2010) for three estrogenic hormones. Although the studies mentioned in this paragraph did not target antibiotics, they

collectively provide evidence that biodegradation can support significant trace pollutant removal during algal WWT.

Algae can directly biodegrade pollutants by heterotrophic metabolism (Neilson and Lewin, 1974; Subashchandrabose et al., 2013, 2011) or extracellular enzymes (Wurster et al., 2003). Algae may also enhance biodegradation indirectly via symbiotic relationships with bacteria, photosynthetically mediated pH changes, or high oxygen production (Muñoz and Guieysse, 2006).

Guo et al. (2015) reported the removal of 90-99.9% of four cephalosporins (supplied at 10 mg L^{-1}) in illuminated *Chlorella pyrenoidosa* cultures against 8.5-27.3% in abiotic control, thus concluding 62-85% of the antibiotics removal was caused by algal activity. Based on preliminary results and the literature, the authors hypothesized algal biodegradation was significant and possibly involved reactive oxygen species formed during photosynthesis.

Dzomba et al. (2015) reported the biodegradation of four tetracyclines (2mg L^{-1}) by algae in sterilised wastewater. An initial phase of fast biodegradation was followed by a slower degradation phase thought to correspond to the degradation of tetracyclines bound to particulate matter. Light conditions were not provided by the authors so the effect of light on the removal kinetics is unknown. Doxycycline was the most susceptible to biodegradation (0.15-3.7 $\times 10^{-2}$ µg $g_{biomass}^{-1}$ d^{-1}), followed by oxytetracycline (0.11-2.9 $\times 10^{-2}$ µg $g_{biomass}^{-1}$ d^{-1}), chlortetracycline (0.17-3.3 $\times 10^{-2}$ µg $g_{biomass}^{-1}$ d^{-1}), and tetracycline (0.013-0.9 $\times 10^{-2}$ µg $g_{biomass}^{-1}$ d^{-1}). Four algae were tested (isolated from hospital and municipal WW), with *Pseudokirchnerilla subcapitata*, *Selenastrum capricornutum* and *Haematoloccus pluvialis* generally supporting stronger biodegradation of the tetracyclines than *Chorella* sp. (this species was consistently associated with the lowest biodegradation reported). These results suggest that

tetracyclines should be degraded by a mixed algal-bacterial biomass in algal WWT, but further testing is needed to determine the role of light in algal biodegradation of tetracyclines.

Since the concentrations of antibiotics found in WW (0.02-30 μg L^{-1} with only a few antibiotics above 1 μg L^{-1}, Michael et al. 2013) are typically well below levels inhibiting microbial activity during biological WWT (antibiotic EC_{50} values to activated sludge microbes range between 20 – 36,000 μg L^{-1}: Halling-Sørensen 2000; Christensen et al. 2006), toxicity should not prevent biodegradation during WWT. This is especially true for eukaryotic algae because these microorganisms are typically 10 times less sensitive to antibiotics than bacteria (antibiotic EC_{50} values to green algae *Selenastrum capricornutum* of 1,000-100,000 μg L^{-1}, De Liguoro et al. 2003; Halling-Sørensen 2000). Unfortunately, the low concentrations at which antibiotics are typically found in WW may also limit their biodegradation, as explained above. For cyanobacteria, the concentrations in WW may nevertheless be high enough to trigger antibiotic biodegradation via detoxification responses and even inhibition. For example, Liu et al. attributed spiramycin biodegradation by *Microcystis aeruginosa* to a detoxification response and reported decreased removal at high initial concentrations due to inhibition (Liu et al., 2015, 2012b). Biodegradation thus removed up to 35% of the antibiotics amoxicillin and spiramycin in a 7 day batch (50 ng L^{-1} to 1 μg L^{-1} initial concentrations).

The biodegradation of some antibiotics by algae may not be significant compared with other removal mechanisms during algal WWT, as reported by de Godos et al. (2012) who concluded tetracycline removal from a laboratory HRAP (dominated by *Chlorella*

vulgaris) was mainly caused by photodegradation and/or sorption. The assays reported in this paper did not fully separate the mechanisms of biodegradation, sorption, and photodegradation, so the conclusion regarding biodegradation is not definitive. In addition, lab test results may not be representative of biodegradation in outdoor systems: Rühmland et al. (2015) thus found that biodegradation in ponds required pollutant contact with sediments, as antibiotics such as trimethoprim and erythromycin were removed well in the pond but not in the *in-situ* quartz tubes lacking surfaces with established biofilm communities. Redox conditions are also important as the same authors showed that sulfamethoxazole degraded better under anaerobic conditions in sub-surface flow wetlands than in open ponds, although most compounds biodegraded better aerobically.

Fungi are uncommon in algal WWT ponds because of the high pH and high nutrient-to-carbon ratios occurring in these systems (McKinney, 2004), but fungi-algal co-cultures for WWT have been tested at laboratory scale in order to improve settling properties (biomass harvesting is one of the main challenge with algae cultivation). The communities were cultivated by maintaining pH around 4.0-5.0 and successfully formed pellets with good settling properties (Zhou et al., 2013b). If implemented at large scale, the presence of fungi may benefit antibiotic degradation because these microorganisms efficiently degrade many organics, including antibiotics, given their abilities to release unspecific extracellular enzymes (Blánquez and Guieysse, 2008; Zhang et al., 2013). However, this system requires continual supplementation (increasing operating costs) and further development.

To conclude, the significance of biodegradation on the fate of antibiotics remains challenging to forecast in algal WWT ponds given to the limited number of biodegradation studies performed under relevant conditions (e.g. low concentrations; Li and Zhang, 2010). The literature suggests algal biodegradation is possible for some antibiotics and that algal-assisted biodegradation could support significant removal (possibly by pH and DO changes, or enhancement of indirect photodegradation – see Section 2.5). Algal WWT ponds have long retention times, highly transient conditions, and support both a high bacterial diversity (Ferrero et al., 2012) and a high level of microbial interaction: all these conditions should benefit the removal of trace contaminants including antibiotics (Guieysse and Wuertz, 2012).

2.5 Photodegradation

2.5.1 Current knowledge of antibiotic photodegradation in conventional systems and surface waters

Various photodegradation mechanisms can significantly contribute to the removal of many pharmaceuticals from surface waters and during advanced oxidation processes [AOPs] (Carstens et al., 2013; Young et al., 2013; Yuan et al., 2011). However, photodegradation is unlikely significant during conventional WWT because of the low light exposure relative to the volume of the system and the high concentration of solids blocking light (Michael et al., 2013). In contrast, algal pond systems allow significant exposure of pollutants to light because they are designed based on surface area and their operation relies upon solar energy for photosynthesis. For further information on the studies of pharmaceutical degradation in surface waters, the recent review by Challis et al. (2014) is recommended.

Photodegradation can occur by direct photolysis or indirect photo-oxidation, depending upon the antibiotic structure, the penetration of light through the water column, and the presence of other molecules and ions in the water matrix. Direct photolysis occurs when light is absorbed by a target molecule and provides the energy to break its chemical bonds. The significance of this mechanism depends on the light absorption spectrum of the antibiotic, which can be influenced by pH, temperature, and interaction with other molecules such as ions and organics (Beliakova et al., 2003; Jiang et al., 2010). Fluorescent molecules (e.g. fluoroquinolone antibiotics) commonly degrade via direct photolysis as a fraction of the photons absorbed induces chemical damage or covalent modification, a mechanism known as photo-bleaching (Herman et al., 2003). Many antibiotics harbour chemical bonds that can be excited by UVA/UVB light but because

other organics present in water also absorb UVA/UVB light strongly (De Lange, 2000), direct photolysis likely occurs only in the top centimetres of the pond water column. This mechanism may still contribute significantly to antibiotic removal if the kinetics are fast enough and if vertical mixing allows frequent pollutant exposure to UV light.

Indirect photodegradation occurs via oxidation by radicals produced when light excites molecules called photosensitizers and produces excited molecules and reactive oxygen species (Davies-Colley et al., 2000a; Kadir and Nelson, 2014). Examples of photosensitizers are organic acids (humic/fulvic acids particularly) and nitrate (Zhao et al., 2013), which are both commonplace in WW influent and effluent. Since photosensitizers usually absorb UV light, indirect photodegradation is increased at the cost of reducing direct photolysis, but direct photolysis is limited to pollutant-specific narrow-wavelength ranges. Indirect photodegradation is less wavelength-dependant and can occur under both UV and visible light as photosensitizers such as chromophoric dissolved organic matter (CDOM) also absorb in the visible spectrum. Not all photosensitizers produce radicals with an equal efficiency: nitrate is considered an inefficient photosensitizer (Mack and Bolton, 1999; Monteiro and Boxall, 2010b), and Sandvik et al. (2000) showed that organic matter in the Mississippi River caused greater rates of singlet oxygen and free radical production than commercial humic acids. Dissolved oxygen, halides, and organic molecules all quench radicals to some degree, as radicals are highly unstable and react quickly with nearby molecules, although other reactive species may be products of the quenching reaction. Therefore, CDOM passively generates and quenches radicals (Glover and Rosario-Ortiz, 2013; Sandvik et al., 2000). In addition, cells produce both natural antioxidants and enzymes that quench radicals and prevent radical damage (Apel and Hirt, 2004).

Table 5: Photodegradation studies on antibiotics.

Antibiotics	Pseudo-first order rate constant [h^{-1}]	Mechanisms mentioned and notes	Reference
Ciprofloxacin	0.02-1.4 (pH 2 - 12)	Photo-oxidation, defluorination, and ring cleavage. Degradation peaked at pH 8, with rates dropping at high and low pH	Wei et al. (2013)
Difloxacin	0.82 (MQ water) 2.0 (river water) Photolysis was fastest at neutral pH	Direct photolysis was dominant. Acetone, hydrogen peroxide, and phosphates increased degradation, while inorganics such as fluoride, nitrate, and sulfate did not influence degradation significantly.	Prabhakaran et al. (2009)
Sarafloxacin	0.26 (MQ water) 1.4 (river water) Photolysis increased with increasing pH		
Sulfamethoxazole Trimethoprim	0.4-0.68 0.03-0.18 (purified water and filtered WW effluent)	Indirect photodegradation led to enhanced degradation in the presence of effluent organic matter, but not in the presence of 'natural organic matter'. Photodegradation rates were highest in filtered WW effluent and lowest in purified water.	Ryan et al. (2011)
Tetracycline	0.32-1.74 [a] 1.7-14.2 (pH 2 - 10)	Low concentrations of dissolved organic matter acted as a photosensitizer, increasing reaction rates.	López-Peñalver et al. (2010)
Chlortetracycline	1.2-3.24 [a] 2.5-37.0 (pH 2 - 8)		
Oxytetracycline	0.77-2.58 [a] 1.7-11.3 (pH 2 - 10)		

[a] Rates varied with initial pollutant concentration.

The kinetics of antibiotics photodegradation are commonly described by pseudo-first order rates (Table 5). The data listed in Table 5 illustrates the dependence of photodegradation kinetics on pH, the water matrix, and the antibiotic structure. Three of the four studies listed in Table 5 reported that the presence of other organic molecules improved antibiotic photodegradation. However, most of these experiments were conducted under low dissolved organics concentrations in order to simulate photodegradation in surface waters and final WWT effluent. It is therefore uncertain if the presence of suspended particles and higher concentrations of dissolved organics may hinder photodegradation during biological WWT in algal ponds.

2.5.2 Potential for antibiotic photodegradation in algal WWT ponds

Because of the strong attenuation of UV light in WW, direct photolysis is less likely to be a dominant photodegradation pathway than indirect photodegradation for antibiotics in algal WWT ponds. Even in maturation ponds, where light penetration is high in comparison to other WWT systems, UVA light is attenuated within 0.07 to 0.24 m of the surface depending on the wavelength (Curtis et al., 1994a). However, longer wavelengths are less attenuated and could generate radicals capable of oxidising antibiotics via interaction with CDOM (see Section 5.3). Even so, Rühmland et al. (2015) determined that photodegradation was limited to the top 10-20 cm of water in a waste stabilisation pond for a selection of photosensitive pharmaceuticals.

Algae release organic molecules (Aota and Nakajima, 2001) which may play a role in enhancing photodegradation via production of hydroxyl radicals, or in inhibiting photo-oxidation by competitive reaction with radicals and attenuation of light. Both radicals and radical scavengers may also be produced by algae cells themselves (Hirayama et al., 1996).

It is thus reported that the presence of washed algae cells in deionised water enhanced photodegradation of the antibiotics ciprofloxacin, norfloxacin, and sulfafadiazine (Zhang et al., 2012a, 2012b; Zhang and Ma, 2013). When Fe^{2+} was present, algae also enhanced photodegradation via the release of photosensitive organic molecules (i.e. CDOM) and the complexation of carboxylic acids (degradation products of the organics excreted by algae) with iron further enhancing hydroxyl radical production by photosensitive Fe^{2+}. The relevance of this research is however limited by the fact a strong UV light source was used rather than full spectrum sunlight, and the use of a

purified water matrix that does not account for the potential effects of interfering substances found in WW.

The specific designs of different types of algal WWT ponds will likely influence how important photo-degradation is in removing antibiotics because of different light penetration in the different systems:

- HRAPs have the highest surface area to volume ratio of all algal WWT ponds, and therefore support the greatest proportional light exposure. Photodegradation is expected to occur for susceptible antibiotics in HRAPs, as suggested by de Godos et al. (2012) in a lab-scale HRAP treating tetracycline-laden simulated WW.
- Facultative ponds have the longest hydraulic retention times of algal WWT ponds, thus compensating for their lower surface area to volume ratios than HRAPs. Facultative ponds also support lower concentrations of biomass than HRAPs, which favours light penetration, but low mixing (especially if temperature stratification occurs) may prevent light exposure to a significant fraction of the antibiotic load.
- Maturation ponds support the lowest concentration of biomass and, consequently, the deepest light penetration of algal WWT ponds (Curtis et al., 1994b). These systems could therefore provide the best WWT process for antibiotic removal if photodegradation is identified as the most important removal pathway.

2.6 Other mechanisms regarding antibiotic removal

2.6.1 Antibiotic hydrolysis in conventional systems

Numerous antibiotics can be deactivated via hydrolysis during conventional WWT and the quantitative significance of this mechanism depends on the pollutant structure. For example, β-lactams are particularly susceptible to hydrolysis (Bell et al., 2011; Längin et al., 2009) while fluoroquinolones and sulphonamides do not break down via hydrolysis under normal environmental conditions (Białk-Bielińska et al., 2012; Kümmerer, 2009a). Hydrolysis can be enzymatically catalysed but this shall be considered as part of biodegradation in this thesis. Besides chemical structure, the rate of abiotic hydrolysis is strongly impacted by pollutant concentration, temperature, and pH (Mitchell et al., 2014; Xuan et al., 2010). Other factors may be involved as, for example, the presence of silica particles catalysing hydrolysis of tetracyclines (Kang et al., 2012).

2.6.2 Potential for antibiotic hydrolysis in algal WWT ponds

In the absence of specific data on antibiotic hydrolysis in algal WWT ponds (possibly due to the difficulty in isolating hydrolysis from other mechanisms), it is only possible to speculate that the mechanisms of hydrolysis are likely similar in conventional and WWT and that the comparative significance of these mechanisms will depend on environmental conditions and HRT. In particular, the shallow design of algal WWT ponds causes these systems to experience considerable diurnal and seasonal temperature variations and, more specifically, high temperatures during the day in summer (Béchet et al., 2011). These high temperatures may enhance the hydrolysis kinetic rates of antibiotics in algal WWT ponds. In addition, because algal WWT ponds can experience large variations in pH, antibiotics that hydrolyse at high pH values may degrade

significantly during the daytime, while antibiotics that hydrolyse at neutral pH values may still degrade during night-time when the pH drops back to neutrality.

2.6.3 Antibiotic conjugation

Apparent increases in antibiotic concentration can be observed during WWT when antibiotics conjugated in the human/animal rumen (Monteiro and Boxall, 2010b) revert back to the original compound during treatment (e.g. sulfamethoxazole, Casas et al. 2015; Kovalova et al. 2012). Conjugates may also be formed during biological WWT, which alters antibacterial properties and uptake rates by bacteria: for example citric-acid conjugates from siderophores excreted by bacteria in order to assist iron uptake (Milner et al., 2014). While no studies specific to algal WWT ponds could be found in the literature, the mechanisms of conjugate formation and/or reversion are likely to be similar during conventional and algae WWT and possibly affected by HRT, pH, DO, and temperature.

2.7 Risk considerations

2.7.1 Degradation products

The generation of toxic degradation products is of high concern during chlorination, ozonation, high intensity UV irradiation, or other oxidation processes (Daugulis et al., 2011). The toxicity of antibiotics' degradation products depends on the degradation pathway and the structure of the parent compound. For example, Guo and Chen (2012) reported the UV irradiation of chlortetracycline could generate more toxic degradation products whereas Kim et al. (2012) showed toxicity decreased during the degradation of sulfamethoxazole and chlortetracycline by UV, electron beam, and ozone. Kang et al. (2012) also reported toxicity decrease during hydrolysis of chlortetracycline, but the hydrolysis products of the similar antibiotics oxytetracycline and tetracycline were more toxic than their parents. Fortunately, biodegradation by diverse communities may remove degradation products during biological WWT, especially in the case of bioavailable and structurally simple antibiotics (Dantas et al., 2008).

2.7.2 Antibiotic-resistance

Antibiotic resistance is encouraged by the presence of antibiotics and is of great concern. In specific cases, the proportion of resistant bacteria has been shown to increase downstream of antibiotic discharge in WW (Akiyama and Savin, 2010; Czekalski et al., 2012; Kristiansson et al., 2011; Pei et al., 2006; Zheng et al., 2011).

Antibiotic resistance can develop in the host (humans or animals) during antibiotic treatment, during WWT, or downstream of WW discharge (Kim and Aga, 2007; Kümmerer, 2009b; Pruden et al., 2013). Biological WWT can increase the risks associated with antibiotic resistance because pathogens carrying resistance-genes are

mixed with large quantities of bacteria adapted to survive in an environment optimized for bacterial growth. This mixing and vigorous growth provide an excellent opportunity for the spread of antibiotic resistance, especially if the presence of antibiotics encourages resistance via selective pressure (Gullberg et al., 2011; Hughes and Andersson, 2012) or through the action of antibiotics as signalling molecules (Allen et al., 2010; Aminov, 2009).

The majority of antibiotic resistance reduction in algal WWT ponds probably occurs via disinfection processes, where resistant pathogens and other bacteria are killed in algal WWT ponds. Maturation ponds especially can provide large reduction in pathogen numbers through a combination of irradiation, high temperature, high DO, high pH, and long retention times (Craggs et al., 2004; Davies-Colley, 2005; Davies-Colley et al., 2000b). Pathogen death does not necessarily remove the threat of resistance genes as these can be transferred to other bacteria prior to or after initial host death (Amabile-Cuevas and Cardenas-Garcia, 1996; Taylor et al., 2011). However, genes outside of cells are more susceptible to damage and degradation by natural mechanisms. For example, Engemann et al. (2006) found that sunlight exposure increased the first-order disappearance rate of selected tetracycline resistance genes 50% above the rate of disappearance under darkness.

To summarize, the mechanisms associated with the occurrence, reduction or encouragement of antibiotic resistance are very complex. Hence, the presence of antibiotics in WW effluents is not always associated with increased resistance downstream of discharge because of the many contributing variables in receiving environments (Cytryn, 2013). In addition, the human health impact of antibiotic resistance downstream of WWT is still uncertain (Boxall et al., 2012).

2.7.3 Wastewater reuse

The use of wastewater for irrigation is highly desirable for agriculture as it provides nutrients (i.e. reduces the need for fertilizers) and reduces fresh water demand (Al-Jassim et al., 2015). However, it is critical to minimize contamination by pathogenic or antibiotic resistant bacteria (Rodríguez et al., 2006). The uptake of antibiotics by plants can hinder plant growth, although the human health risks associated with the consumption of antibiotic-containing plants are uncertain (Becerra-Castro et al., 2015; Gothwal and Shashidhar, 2014; Jjemba, 2002).

In addition, the release of antibiotic-laden wastewater into freshwater ecosystems can lead to the presence of antibiotic on drinking water sources, which is an issue as demand for water sources increases (Brookes et al., 2014; Zhang et al., 2014). Antibiotics have indeed been detected in drinking water (Gabarrón et al., 2016; Kümmerer, 2009a; Stackelberg et al., 2004), although the human health risks associated with this occurrence are uncertain at the minute concentrations involved (Boxall et al., 2012).

2.8 Conclusions and research objectives

While direct evidence of antibiotic removal during algal WWT is very limited, cross-analysing literature on the mechanisms of antibiotic removal in conventional WWT against broader knowledge of pollutant removal during algal WWT suggests that the unique ecological and environmental conditions (e.g. algal biomass, light, pH, temperature) found in algal WWT ponds should have a profound and specific impact on the mechanisms causing antibiotic removal in these systems. In particular:

- Long HRTs in algal WWT ponds may allow removal mechanisms with slow kinetics to become significant. Significant hydrolysis of antibiotics prone to this mechanism may therefore occur in algae ponds. Hydrolsis, sorption, biodegradation, and photodegradation are known to be influenced by temperature, pH, and DO during conventional WWT, and these parameters vary significantly in algal WWT ponds.
- While antibiotic biodegradation may be affected by algal activity, there is currently little research to quantify these effects. Some algae have the ability to biodegrade some antibiotics and other pollutants, but biodegradation in full scale systems also depends on many other factors.
- There is also little research to quantify antibiotic sorption to algal biomass in WWT systems, yet the sorption of antibiotics to biomass may be different with eukaryotic cells (algae) vs. prokaryotic cells (bacteria), and sorption may also be affected by differences in extracellular polymeric substances produced by algae vs. bacteria.

- The large surface-area-to-volume ratios of algal WWT ponds increases the significance of photodegradation mechanisms compared to conventional WWT and the photodegradation of some pollutants has been demonstrated in algal WWT ponds. Photodegradation is therefore likely to be a significant removal mechanism during algal WWT, but photodegradation requires further investigation in the presence of high concentrations of dissolved and suspended solids relevant to algal WWT ponds.
- Indirect photodegradation mechanisms should dominate over direct photolysis in algal WWT ponds due to the presence of organic compounds absorbing and scattering light.
- Light-based mechanisms may also reduce the risks associated with antibiotic resistance in algal WWT through disinfection processes and the destruction of antibiotic resistance genes.

Based on these conclusions, the following research hypotheses and overall research objectives were formed, as previously outlined in the introduction (Section 1.3), using a case study of TET removal in HRAP:

Research hypotheses: The fate of antibiotics in algal WWT will be affected by photo-based degradation pathways and the diurnal variation in pH and DO caused by photosynthetic activity. The characteristics of sorption and biodegradation in algal ponds will be significantly different than during conventional biological treatment due to the presence of photosynthetic microorganisms. The understanding of the fate of antibiotics during algal WWT will enable the optimisation of algal WWT processes for the removal of antibiotics.

Research objectives, with specific research questions listed under each objective:

1. To determine how efficiently TET is removed from WW in HRAP systems, and how sensitive TET removal is to changes in scale, operation, and environmental conditions.
    - Is TET-containing WW passing through HRAP systems likely to pose a high risk to environments downstream, or is TET sufficiently well-removed in HRAPs that the risk of TET affecting environments downstream is low?
2. To determine the relative significance of potential TET removal mechanisms such as photodegradation, biodegradation, sorption, and hydrolysis.
    - Does algae activity affect TET removal directly via biodegradation or sorption onto the biomass, or do abiotic degradation processes such as photodegradation and hydrolysis dominate the TET removal?
3. To identify any potential links between TET removal rates in the presence of algal-bacterial biomass and the monitored environmental and WW conditions.
    - Does algae activity affect pollutant removal indirectly via pH and dissolved oxygen concentration fluctuations?
    - If the TET removal kinetic rates depend on temperature changes or sunlight intensity (e.g. for photodegradation), how are seasonal changes likely to affect TET removal in algal WWT ponds?
4. To predict potential TET removal in a variety of algal pond designs.
    - Is TET removal sensitive to design differences in a variety of algal ponds, or is the overall removal similar?

The scope of the research investigation was limited to exclude investigation of the antibiotic degradation products, toxicity, and antibiotic resistance, since the analytical

ability needed was not readily available, and there was insufficient time to consider each of these subject areas adequately.

3. Methodology

This section first describes the overall strategy of the experimental work, based on the objectives outlined in the conclusion of the literature review (Section 2.8). Then, a justification is made for the selection of TET and high rate algal ponds (HRAP) among the other possible antibiotics and algal pond designs that could have been studied. The following sub-sections then describe the experimental designs and analytical methodologies used to research the subject.

3.1 Research strategy

Indoor lab scale HRAP studies were used to investigate the overall fate of TET, and the efficiency of TET removal. The lab scale HRAPs were operated under five different operating conditions, varying HRT and light supply to assess TET removal a wide range of conditions. Outdoor pilot scale studies conducted under Oceanic (NZ) and Mediterranean (Spain) climates were then used to validate the lab-scale findings, especially considering the differences in scale and the effects of sunlight vs. artificial light (Objective One).

Batch experimentation was used to clearly distinguish the various mechanisms of removal, using repeated tests with biomass and HRAP effluent sourced from the HRAPs operated continuously under different conditions (resulting in different biomass concentrations, ecology, and effluent characteristics). Initial findings regarding TET removal mechanisms using batch tests under artificial light were validated under outdoor conditions, and with several TET pulse tests in the pilot HRAPs (Objective Two).

The pulse TET experiments in the pilot HRAPs were also used to investigate potential links between TET removal and the monitored environmental and WW conditions. In addition, batch tests were performed with adjusted pH or different suspended solids concentrations, to investigate the effects of changing these conditions upon TET removal (Objective Three).

A kinetic model was developed to investigate whether TET removal could be accurately predicted in the pilot HRAPs, based on parameters obtained from batch experiments. This model was then used to estimate the potential TET fate in other algal WWT ponds (Objective Four).

3.2 Constraints

3.2.1 Selection of tetracycline as an example antibiotic

Tetracycline was chosen as the antibiotic of interest for the following reasons:

- It is commonly used for both animal and human treatment (MAF 2011)
- It has been detected in surface waters (Sarmah et al., 2006)
- It was detectable by analysis with high performance liquid chromatography (HPLC) using diode-array-detection (DAD), which was available in our laboratory, since TET has two UV absorption peaks at 270 and 360 nm.
- It may potentially be removed by multiple mechanisms
    - TET is generally reported as non-biodegradable under both aerobic and anaerobic conditions (Cetecioglu et al. 2013). However, the algal-bacterial community may together be able to biodegrade TET (Dzomba et al., 2015). TET is soluble, and is designed to targets proteins inside

cells, therefore TET should be available for biodegradation by microbes; the only limitation for TET biodegradation should be the genetic ability and incentive for the microbes to target the TET for degradation (Section 2.4.2).

  - TET is fluorescent under some conditions, and therefore may be sensitive to direct photodegradation (particularly at high pH; Pena et al. 1998).
  - TET harbours numerous dipoles and positions that radical oxygen species can attack, so indirect photo-degradation could be important.

- Antibiotic resistance to TET is widespread, so there is less ethical concern about the experimentation itself having a major impact on antibiotic resistance levels.
- Most of the typical degradation products of TET are less toxic than TET (Halling-Sørensen et al., 2002) so the risks of toxic by-products formation is low.[a]

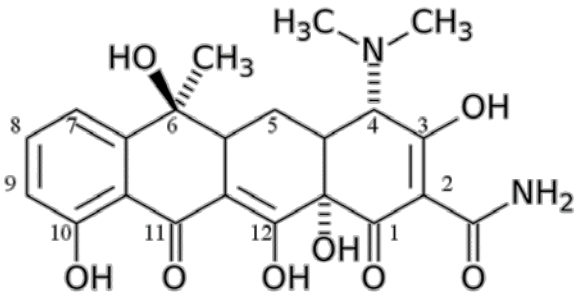

Figure 1: Structure of tetracycline

- There are three functional groups in the TET molecule (Figure 1), two of which with pKa values in the range of daily pH fluctuations reported in algal WWT ponds (pKa values 7.8, and 9.6; Qiang & Adams 2004). Therefore, since we know that the antibiotic structure will change with pH, diurnal changes in pH are likely to affect antibiotic removal.

To facilitate sample analysis, experimental work began using high antibiotic concentrations (mg L^{-1}), and later experimental work validated these findings using

[a] Suitable analytical equipment for identifying toxic degradation products was not available (see Section 3.6 for analytical methods).

lower antibiotic concentrations (µg L^{-1}). The physical and chemical parameters of TET are reported in Table 14, Section 3.6.

Although TET is the focus of the experiments, some final experiments did consider four other antibiotics (see Section 3.5), in order to understand whether the experimental methods used in this thesis for the study of TET could be easily applied to the study of other antibiotics, and whether similar patterns of removal mechanisms were observed.

3.2.2 Selection of HRAPs as an algal WWT system for experiments

HRAPs support higher algal biomass concentrations and shorter retention times than facultative and maturation ponds. Due to the mixing of the paddle wheel and the shallowness of the ponds, the environmental conditions and suspended solids concentrations in HRAPs are also more homogenous across depth and width than facultative and maturation ponds. This well-mixed[a] nature of the HRAPs enables simpler modelling and HRAPs are much easier to mimic at laboratory and pilot scale.[b] The shallow HRAP depth also concentrates algal productivity into a smaller volume than facultative and maturation ponds, causing higher biomass concentrations and greater fl1qauctuations of pH, temperature and DO concentrations. Therefore, the effects of these diurnal fluctuations should be more evident in HRAPs than in facultative and maturation ponds, and the higher biomass concentrations increase the likelihood of observing sorption and biodegradation effects. HRAPs were therefore used as a starting point for research, the results of which were then applied to the consideration of other algal WWT ponds.

[a] Although large HRAPs do have some similarity to a plug-flow reactor with recycle as the WW moves around the raceway, they can be considered well-mixed since the frequency of mixing is high relative to the hydraulic retention of the pond (e.g. water may take around an hour to complete a circuit, but the HRT is around 7 days). At lab and pilot scales, this well-mixed nature is accentuated further, as the raceway length is reduced compared to the area occupied by the paddle wheel. Turbulent mixing in the lab-scale and pilot scale HRAPs was confirmed visually by observing the algal flocs moving within the ponds.

[b] In this thesis, 'lab-scale' was 7 L, and 'pilot scale' was 180-900 L.

3.3 Design and operation of HRAP systems

3.3.1 Lab-scale HRAP: 7 L

The purpose of the lab-scale HRAPs was to provide a first look at TET removal under conditions that were as relevant as possible to outdoor systems, while controlling light supply and ambient temperature to facilitate reproducible experimental conditions.

de Godos et al. (2012) also used a 7 L HRAP to investigate TET fate in algal WWT: the HRAP used by de Godos et al. (2012) used a liquid depth of 0.2 m, a continuous light intensity of 10 W m^{-2} PAR, provision of UVA/B light using a reptile tube in addition to cool daylight fluorescent tubes, and once-daily feeding of synthetic WW. The design for the HRAP in the current thesis was chosen to increase the relevance to outdoor conditions: 1) real WW was used instead of synthetic WW; 2) to compensate for the low light intensity used in our lab the working liquid depth chosen was reduced from 0.2 m depth to 0.065 m depth; 3) full-spectrum fluorescent tubes were chosen instead of supplementing cool-daylight tubes with reptile UV tubes, so that consistency in light spectrum was maintained in all areas of the HRAP and so lighting conditions could be replicated in the batch experiments; 4) a diurnal light cycle was used to reproduce the fluctuations in pH and DO concentrations observed outdoors.

Two stainless steel lab-scale HRAPs (Figure 2: LxWxD = 0.5 m x 0.3 m x 0.12m) with an average working volume of 7 L (0.065 m liquid depth) were continuously mixed by paddle wheels (6 blades) at 28 rpm to maintain a centre-channel liquid velocity of 0.1 m s^{-1}. This system has previously been used by other researchers (Alcántara et al., 2015). Light was provided above the HRAPs by 9 × 36 W Viva-Lite® T8 fluorescent tubes. The average photon flux was 160 µmol m^{-2} s^{-1} PAR using a hand-held light meter. Because the light intensity provided by the fluorescent tubes varied at different

positions across the HRAP surface, actinometry was used to better quantify the average light intensity. Based on the actinometry test conducted (see Appendix S4.1) the average light intensity received was 46 W m^{-2}. Measurement by photoradiometer found that the ratios of UVA, UVB, and PAR light intensity were 7.1%, 0.05%, & 92.8% respectively. These lighting conditions and HRAP design were chosen to provide a similar light energy/volume ratio as might be found outdoors in summer for a 0.2 m deep HRAP (Volumetric light supply was ~ 700 W m^{-3} for the lab-scale HRAPs, and daily maximum sunlight intensity was recorded from 70-400 W m^{-2} PAR March-May 2015, Palmerston North NZ, which equates to 350-2,000 W m^{-3}).

The HRAPs were operated under 4 or 7 days HRT, with 8, 12, or 16 hours of light illumination per 24 hours (See Table 6). Primary-clarified WW was collected from Palmerston North City Council Totara Road WWT Plant (PNCC-WWTP), and stored frozen below -10°C until required. The characteristics of the WW are shown in Table 7. Freshly defrosted WW kept below 6°C was supplied to the HRAPs using a peristaltic pump (Watson Marlow 120S), using pulses of 5 min every 90 min or 1 min every 20 min (see Table 6) to enable precise control over daily flow-rates. Purified (RO) water was supplied with the influent to compensate for evaporation. Effluent was removed once daily.[a] TET was either spiked in the influent WW, or supplied with the evaporation-make-up water using a separate peristaltic pump (Thermo Fisher Model 77122-24) (see Table 6), for an influent concentration of 2 mg_{TET} L_{WW}^{-1}. The lab-HRAPs were inoculated with algal-bacterial biomass from Rongotea primary and secondary facultative ponds[b].

[a] An effluent overflow was initially trialled to have a continuous removal of effluent, but problems developed with large biomass flocs not exiting the HRAP in the effluent, and the once-daily removal of effluent was used.

[b] Roughly equal volumes of the primary and secondary facultative pond were mixed and used to inoculate the HRAPs to ensure diversity of species were present. The Rongotea waste stabilization pond (WSP)

To confirm the relevance of the experimental design to full-scale, pond performance (productivity, TOC, and dissolved nutrients), biomass concentrations, and environmental conditions (pH, DO, temperature) were monitored regularly, using analytical techniques described in Section 3.7. The aqueous TET concentrations and sorbed TET concentrations in the HRAP were monitored as described in Section 3.6.

system services ≈600 people equivalent. WSP biomass was collected from the top 20 cm of the ponds to gather a high concentration of algae, and the biomass was concentrated 3-fold by 5 min centrifugation at 4,000 g before addition to the HRAPs.

Table 6: Operational stages of the lab-scale HRAPs

Name	Date period started (2014)	HRT [d] [a]	Light supply /24 h	Influent pump timing [b]	TET Addition [c]
Stabilization	25th Jun	7	12	5 / 90 min	None
Initial trial with effluent overflow.	9th Jul	7	12	5 / 90 min	None: HRAP-A in WW: HRAP-B
Stage I	29th July	7	12	5 / 90 min	Both HRAPs: in WW
Stage II	28th Aug	4	12	5 / 90 min	Both HRAPs: in WW
Transition & rotifer treatment	16th Sep	7	12	5 / 90 min	Both HRAPs: in WW
Stage III	9th Oct	7	8	5 / 90 min	Both HRAPs: in WW
Transition Period to change pumping regime.	23rd Oct	7	8	1 / 20 min	Both HRAPs: in RO water
Stage IV	30th Oct	4	8	1 / 20 min	Both HRAPs: in RO water
Stage V	30th Nov	4	16	1 / 20 min	Both HRAPs: in RO water
Terminated	18th Dec				

[a] The SRT is equal to the HRT under well-mixed conditions without recycle, which were assumed for the design of the HRAPs.
[b] To maintain a consistent low flow-rate, the pump was put on a timer to provide regular pulses of influent throughout the day – in early stages the pump was on for 5 min every 90 min, and later this was adjusted to the pump turning on for 1 min every 20 min.
[c] 'in WW': TET was added to the influent WW; 'in RO water': TET was added with the purified water (reverse osmosis, RO) supplied to the HRAP to compensate for evaporation. See Appendix Section 4.2 for TET stability in water.

Table 7: Wastewater characteristics for the lab-scale HRAPs.

Data reported as mean ± st.dev. (n)

Characteristic [mg/L]	Stages I to III	Stages IV and V
COD[a]	290 ± 12 (2)	239 ± 38 (4)
BOD[a]	131 ± n.a. (1)	123 ± n.a. (1)
TOC[a]	95 ± 12 (10)	75 ± 25 (20)
TN[a]	51 ± 6 (10)	52 ± 10 (20)
IC[a]	51 ± 2 (10)	22 ± 3 (20)
Dissolved N-NO_2^-	2.1 ± 0.1 (5)	2.1 ± 0.1 (9)
Dissolved N-NO_3^-	0 (5)	0 (9)
Dissolved P-PO_4^-	4.0 ± 0.8 (5)	1.2 ± 0.8 (9)
TSS[a]	91 ± 7 (6)	60 ± 2 (4)
VSS[a]	60 ± 3 (2)	67 ± 2 (4)

[a] Definition of acronyms: COD: Chemical oxygen demand; BOD: Biological oxygen demand; TOC: Total organic carbon; TN: Total nitrogen; IC: Inorganic carbon; TSS: Total suspended solids; VSS: Volatile suspended solids.

Figure 2: The lab-scale high rate algal ponds (HRAP) used in continuous studies

3.3.2 Pilot-scale HRAP: 900 L

The purpose of this 900 L HRAP was to validate findings from the lab-scale HRAP experiments, with increased relevance to full scale.

The concrete pilot-scale HRAP (Figure 3: L × W × H = 3 m × 1.1 m × 0.5 m) with a working volume of about 900 L (working liquid depth 0.26 m) was continually mixed with a paddle wheel (4 blades) at 11 rpm, giving a centre-channel velocity of 0.16 m s^{-1}. The HRAP was situated at PNCC-WWTP, inoculated in Dec 2014, and experiments occurred from Jan to Apr 2015. Primary settled WW was supplied continuously from the WWTP at 9 day HRT using a peristaltic pump (Cole Palmer 7553-75). This 9 day HRT is slightly longer HRT than the lab-scale HRAPs, and was chosen as we wanted to minimise the risk of the HRAP overflowing in between sampling times if the outlet was blocked. Another peristaltic pump (Thermo Fisher Model 77122-24) was used to supply TET stock solution to the HRAP in pulses (5min / 90min, digital timer). The TET stock solution (200 mg L^{-1}, stored at room temperature) was freshly prepared every 3-4 days. The HRAP effluent overflowed into an outlet pipe positioned mid-channel.

The HRAP was inoculated with 200 L of algal-bacterial biomass from Rongotea primary and secondary facultative ponds (50:50 vol. ratio). During all experimental work (Feb-May 2015), a 9 day HRT (100 L d^{-1} influent flow) was maintained.

Because the HRAP was situated at a WWT plant, fresh WW was sourced from a WW channel directly after primary treatment. The WW was monitored regularly, with typical characteristics reported in Table 8.

Pond performance (TOC and dissolved nutrients), biomass concentrations, and environmental conditions (pH, DO, temperature) were monitored regularly using analytical techniques described in Section 3.7. The concentration of TET in the pond

and concentration of TET sorbed to the biomass were monitored as described in Section 3.6. The monthly average weather conditions are shown in Table 9. For removal efficiency calculations, since effluent flow-rates for the HRAP were not measured directly, effluent flow-rates were estimated based upon influent flow-rate and the mean monthly net evaporation (evaporation – rainfall) from the weather station data (Table 9).

Table 8: Wastewater characteristics for sampled wastewater during 900 L HRAP operation Feb-April 2015. Values reported as mean ± st.dev. (n)

Characteristic	**[mg L^{-1}]**
COD[a]	357 ± 128 (3)
TOC[a]	98 ± 23 (17)
TN[a]	31 ± 10 (17)
IC[a]	52 ± 33 (17)
Dissolved N-NO_2^-	0.52 ± 0.78 (13)
Dissolved N-NO_3^-	0.2 ± 0.4 (13)
Dissolved P-PO_4^-	2.0 ± 0.4 (13)
TSS[a]	92 ± 28 (31)

[a] Acronyms are defined in the footnote for Table 7

Figure 3: The 900 L Pilot scale HRAP operated at PNCC-WWTP

Table 9: Monthly weather conditions during experimentation in Palmerston North. [a]

	Units	Dec-14	Jan-15	Feb-15	Mar-15	Apr-15
Mean air temperature	°C	16.9	19.6	17.8	18.0	14.8
Mean daily max air temperature	°C	21.5	25.6	23	23.15	19.2
Mean daily min air temperature	°C	12.3	13.5	12.7	12.7	10.4
Total sunshine	h	167.9	287.7	203	195.9	140.7
Mean daily global radiation	MJ m^{-2}	21.3	26.3	20.8	15.8	10.4
Mean wind speed	m s^{-1}	4.1	3.8	4.05	3.05	3.25
Mean of 9am relative humidity	%	77.5	72.6	77.2	85.7	84.5
Mean daily open water evaporation	mm	3.89	5.54	4.65	3.26	2.36
Mean daily rainfall	mm	2.95	0.38	2.79	2.28	6.73

[a] Data retrieved from clifo.niwa.co.nz on 11th May 2016, with data averaged from two weather stations (Agent numbers: 3243 & 21963) in Palmerston North

Two pulse tests were performed in the 900 L pilot HRAP, spiking TET into the HRAP at high concentration to assess the difference between TET removal during the night and the day. For this purpose, 2 g TET powder was added into the 900 L HRAP at approximately 8 pm (after sunset), resulting in a theoretical increase of 2.2 mg L^{-1} TET concentration in the HRAP.[a] The influent TET flow (but not the WW flow) was stopped for 48 hours from the beginning of the pulse test. Initial samples were taken 15 min after the TET was added to allow the TET time to disperse evenly throughout the HRAP. Monitoring continued the following morning before sunrise, at approximately 7 am, with regular monitoring every hour afterwards. The pH, DO, PAR light intensity, and temperature were data-logged during the pulse experiment. TSS concentration was measured at 4 regular intervals.

[a] The TET powder was introduced through a sieve to improve rapid dissolution. Solubility was not an issue at the concentrations involved (see Table 14 Section 3.6), so the powder should have dissolved fully within a few minutes.

3.3.3 Pilot-scale HRAP: 180 L

A 180 L HRAP was used to validate previous findings under different climatic conditions and at lower TET influent concentrations. Different equipment availability at University of Valladolid and the scale of the pilot HRAP enabled better monitoring of the effluent flows, more consistent influent composition, and different HRAP design.

The pilot HRAP was constructed in PVC (LxWxD = 1.7 m x 0.82 m x 0.25), with a central wall to form two channels and mid-channel baffles at each end to improve the flow efficiency (Figure 4). A paddle wheel (6 blades, 10.5 rpm) was used to support a mid-channel velocity of 0.2 m/s. The HRAP was located on the roof of a small shed at University of Valladolid. Shading from nearby buildings and trees limited direct sunlight from ~10 am to ~4:30 pm. The HRAP was inoculated with 1.5 L of settled biomass (23 g_{TSS} L^{-1}) taken from another pilot HRAP (Posadas et al., 2015b).

There were four stages of pilot-HRAP operation (Table 10).[a] The HRAP was operated at 7 day HRT for 52 days (Stage I) to establish a baseline HRAP performance. Still at 7 day HRT, TET was then supplied in the influent WW for 35 days (100 µg TET L^{-1}; Stage II). For Stage III, the influent WW flow was increased to operate the HRAP at 4 days HRT, with the same 100 µg TET L^{-1} for 17 days. The 4 day HRT was maintained during Stage IV for another 27 days, with TET pulse tests performed as described in the next section.

Influent was collected every 1-2 days as required from a pilot-scale primary WWT operation (1 mm rotary sieve and primary sedimentation) that sourced WW from the municipal sewer running past the university. WW characteristics were measured twice weekly and are summarised in Table 10. The WW was stored in a 60 L plastic drum

[a] Each stage was monitored during a period of time equivalent to at least four HRTs, and HRAP performance (TSS concentration, COD removal, and nutrient concentrations) was monitored to ensure the HRAP was reasonably stable before moving to the next stage.

kept at 4-6°C, and pumped continuously into the HRAP at the required HRT using a peristaltic pump (Masterflex Console Drive 77521-57). TET stock solution (100 mg L^{-1} prepared weekly) was added to the influent WW to obtain the desired concentration. The 60 L drum containing the influent feed was mixed using a submergible aquarium pump. Effluent overflowed from the HRAP through a pipe into the shed below and clarified in an 8 L clarifier, out of direct sunlight. Clarified effluent was collected in a 100 L drum, and weighed to quantify the average daily flow. Sediment biomass was removed from the clarifier twice weekly. The monthly average weather conditions are shown in Table 11.

Table 10: Wastewater characteristics during 180 L HRAP operation April-Aug 2015

	Stage I	**Stage II**	**Stage III**	**Stage IV**
HRAP conditions	Start-up (no TET)	Continuous TET	Continuous TET	Continuous or Pulsed TET
HRT	7	7	4	4
TSS[a] [g/L]:	0.12 ± 0.03 (6)	0.13 ± 0.02 (10)	0.11 ± 0.05 (3)	0.12 ± 0.04 (8)
VSS[a] [g/L]:	0.11 ± 0.03 (5)	0.12 ± 0.01 (10)	0.10 ± 0.04 (3)	0.10 ± 0.04 (8)
COD[a] [mg/L]	787 ± 392 (6)	621 ± 63 (8)	669 ± 258 (3)	690 ± 104 (8)
TOC[a] [mg/L]	176 ± 44 (6)	165 ± 11 (9)	147 ± 113 (3)	171 ± 19 (8)
TN[a] [mg/L]	107 ± 26 (6)	102 ± 9 (9)	70 ± 20 (3)	93 ± 11 (8)
IC[a] [mg/L]	87 ± 19 (6)	87 ± 17 (9)	82 ± 7 (3)	88 ± 9 (8)
N-NH_4^+ [mg/L]	43.7 ± 12.4 (6)	51.1 ± 9.7 (10)	55.6 ± 1.0 (3)	45.9 ± 11.8 (8)
Dissolved N-NO_2^-	not detected (6)	not detected (10)	not detected (3)	not detected (8)
Dissolved N-NO_3^-	not detected (6)	not detected (10)	not detected (3)	not detected (8)
Dissolved P-PO_4^-	3.1 ± 0.7 (6)	3.3 ± 0.1 (10)	3.3 ± 2.1 (3)	2.6 ± 0.5 (8)

[a] Acronyms are defined in the footnote for Table 7

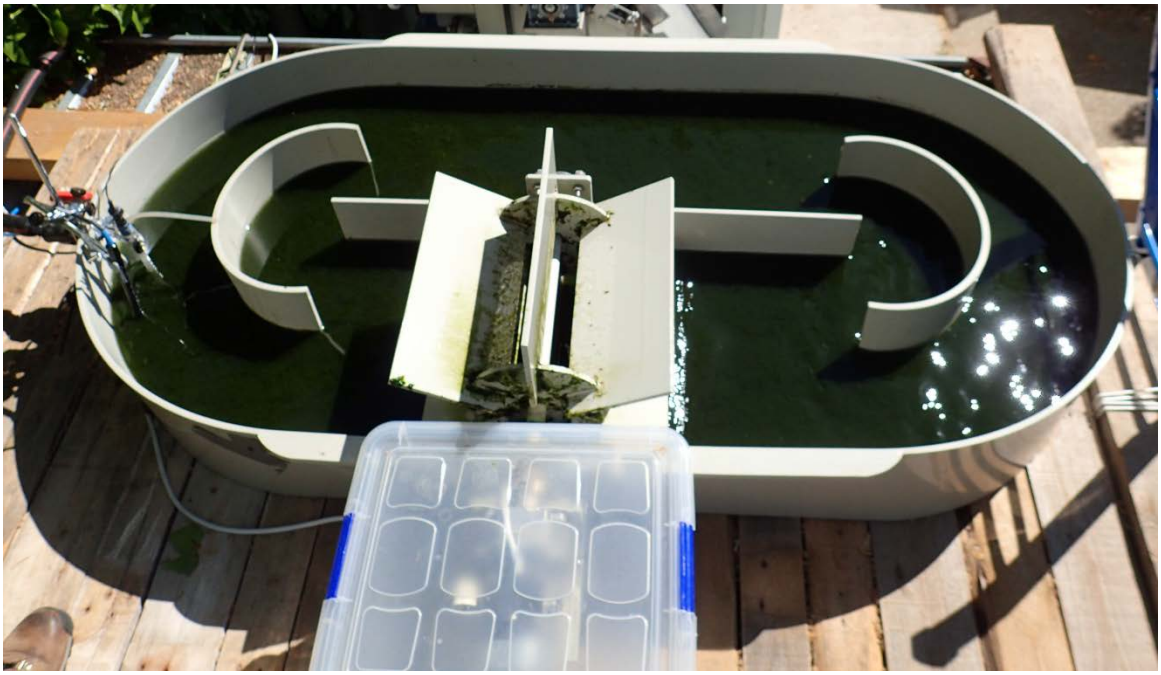

Figure 4: The 180 L pilot-scale HRAP operated at University of Valladolid, Spain

Table 11: Average weather conditions during the 180 L HRAP operation. [a]

	Apr-15	May-15	Jun-15	Jul-15	Aug-15
Mean daily humidity [%]	66	53	49	40	44
Mean daily wind speed [km h^{-1}]	13	16	12	12	12
Max daily temperature [°C]	17	22	27	31	29
Mean daily temperature [°C]	11	15	19	22	20
Min temperature [°C]	5	7	11	14	13
Total monthly precipitation [mm]	22	10	57	0	1

[a] Data taken from www.wunderground.com, retrieved 9 Oct 2014, from measurements at Valladolid Airport.

Pond performance (productivity, COD, TOC, and dissolved nutrients), biomass concentrations, and environmental conditions (pH, DO, temperature) were monitored regularly (twice weekly), using analytical techniques described in Section 3.7. The aqueous concentration of TET in the pond was measured by solid phase extraction (SPE), and the concentration of TET sorbed to the biomass was monitored as described in Section 3.6.

Several pulse tests were performed in the 180 L pilot HRAP, spiking TET into the HRAP at high concentration in order to observe differences between TET removal during the night and the day. Before each pulse experiment, two samples were taken for analysis of the baseline TET concentration. The TET-spiked influent WW flow was stopped immediately before each pulse experiment, and the HRAP was instead supplied with fresh WW without added TET for the duration of the experiment. TET pulses were supplied by closing the HRAP outlet, adding 180 mL of 100 mg L^{-1} TET stock solution (theoretically increasing the HRAP TET concentration by 100 μg L^{-1}), and mixing the HRAP for 5 min before outlet was re-opened and the TET concentration was measured. Four experiments were conducted with 2 TET pulses in a single day. In these experiments, the first pulse was supplied in the morning (normally between 10:30 and 11:30 am), and TET concentrations were monitored until the HRAP was fully shaded in the evening. A second pulse was then begun (normally at 7:30 pm), and TET concentrations were monitored for every half hour for 90 min, and then monitored regularly the following day starting at 7-8 am (near sunrise) until late afternoon. After the pulse monitoring was completed, the TET was added to the influent feed tank again. Two additional pulse experiments were conducted with a single pulse supplied. One of these replicated the morning pulse above, but did not continue with the evening TET pulse; the second replicated the evening pulse above, without a prior morning TET pulse that day. The pH, DO, temperature, and light intensity were recorded continuously using data-loggers.

3.4 Batch experiments with tetracycline

The batch experiments described in the subsections below are divided into two main groups: 1) 100 mL batch tests performed during experimentation with the lab-scale HRAP operation in NZ, and 2) 2.5 L batch tests performed during experimentation with the pilot-scale HRAP operation in Spain. These batch experiments were conducted to distinguish the various mechanisms of removal, and to observe the effects of changing parameters such as TSS and pH independently, otherwise maintaining conditions as similar as possible to the HRAP operation.

Several preliminary batch trials were also performed to test and improve experimental designs, including photodegradation and biodegradation tests in closed glass flasks, photodegradation tests in 10 mL tubes, and photodegradation tests in phosphate buffered solution in 100 mL flasks.

3.4.1 100 mL Batch tests

Indoor batch tests were conducted to identify the main mechanisms of TET removal using biomass sourced from the two lab-scale HRAPs in NZ (A & B, Section 3.3.1). Biomass was collected at the end of the dark period in the HRAPs' diurnal cycle. Dead biomass was prepared by autoclaving biomass at 121 °C for 20 min. Batch tests were conducted in tall form 150 mL beakers, with 100 mL of WW or MQ water, and 2 mL of TET stock solution [100 mg L^{-1}] (see Table 12). The beakers were incubated in a temperature controlled environment at 25 ± 1°C (Contherm 1400 cooled incubator), continually mixed using a rotational shaker (IKA®KS basic) at 150 rpm. For batch tests incubated under light conditions, the light intensity was 15.9 ± 2.0 (st.dev.) W m^{-2} (Viva-Lite® 18 W T8 tubes). Batch tests incubated under dark conditions were covered in foil. The light intensity received by the beakers was one-third of the surface light

intensity received by the HRAPs, although liquid depth was also slightly reduced (46 mm in beakers compared with 60-70 mm in HRAPs).

Table 12: Design of experiment for standard batch trial

	Pond A		Pond B		MQ water	
Active Biomass	1. Light	6. Dark	3. Light	8. Dark		
Dead Biomass	2. Light	7. Dark	4. Light	9. Dark		
Controls					5. Light	10. Dark

For TET analysis, 3 mL samples were taken and immediately filtered through a 0.22 µm Millipore syringe filter (mixed cellulose esters). The first mL of filtrate was discarded and the next mL was saved for HPLC analysis. Sampling times were 0, 4, 8, and 24 hours under light conditions, and 0, 1, 3, and 6 days under dark conditions.[a] Evaporation was compensated by recording the beaker weight after each sampling time and at the next sampling time MQ water was added to achieve this weight before samples were taken for TET analysis.

The initial TSS concentration was recorded for each biomass type (A & B, active and autoclaved), and at the end of the analysis period the pH, DO, and temperature were measured.

For one batch experiment, the above procedure was adapted in order to vary the TSS concentration of the biomass and to provide 50% WW as an additional food and nutrient source. In this batch experiment, duplicates were prepared with centrifuged HRAP biomass (4,000 rpm for 10 min in 500 mL tubes, Sigma 6-16S centrifuge). The HRAP biomass was resuspended in MQ water at 2x the desired concentrations, with a volume of 50 mL. A further 50 mL of WW was then added to result in the final desired

[a] Different timing regimes were used because pre-trials indicated that the degradation of TET was much faster in the presence of light than under darkness

concentration (0.1, 0.25, or 0.5 gTSS L^{-1}). Three controls with 50% WW in MQ water were also prepared. TET stock solution (2 mL of 100 mg L^{-1}) was added and the beakers were incubated for 24 hours at 25 ± 1°C. One WW control was covered in foil to maintain dark conditions, and all other beakers were incubated in the light (as above). TET sampling then proceeded as normal.

3.4.2 2.5 L Batch tests

During research in Spain, batch tests were again used to investigate the TET removal mechanisms with biomass from the 180 L pilot-scale HRAP. The size of the batch experiments were increased from 100 mL to 2.5 L, so that the batch tests could be performed with a similar liquid depth as the HRAP (150 mm deep), and thus maintain similar light attenuation in the batch tests compared with the HRAP when exposed to sunlight.

The effect of diurnal light exposure, pH and sorption to biomass upon TET removal was therefore studied in 'batch reactors': 2.5 L plastic open-top stirred-tanks (185 mm surface diameter and 145 mm liquid depth; Figure 5). Active biomass for the experiments was sourced from the 180 L pilot HRAP and strained through a colander (3 mm holes, to remove debris) before use. Dead biomass was prepared by autoclaving biomass at 121°C for 20 min and cooling to room temperature. All batch reactors were mixed at 250 rpm (Thermo Scientific 15-position magnetic stirrers) during experiments. TET stock solution (100 mg L^{-1}) was added and manually mixed for 30 seconds before each initial sample was taken. At each sampling time the TET concentration (10 mL samples taken), pH, light (PAR), DO concentration, and temperature were monitored. TSS concentrations were measured for each batch reactor at the end of the experiment.

Figure 5: Outdoor full-day batch test in 2.5 L batch reactors. Conditions are, clockwise from top left: autoclaved 100% HRAP biomass, 10% HRAP biomass in MQ water, filtrate, MQ water, 10% HRAP biomass in effluent, and 100% HRAP biomass.

3.4.2.1 Full-day batch tests outdoors

Full day outdoor batch trials were conducted to distinguish the different mechanisms of TET removal during diurnal light exposure.

To study the effect of diurnal light exposure, full day outdoor batch trials were conducted with 2.45 L of one of the following mixtures as shown in Table 13: active biomass, autoclaved biomass, 10% active biomass in effluent, 10% active biomass in MQ, effluent filtrate, and 100% MQ water. Effluent filtrate was prepared using 0.7 µm glass fibre filters. The initial two experiments used two replicates with active biomass, one replicate with autoclaved biomass, and one MQ water control. Later experiments examined single replicates of each condition listed in Table 13. The batch reactors were positioned beside the 180 L HRAP to maintain the same lighting conditions. After TET stock solution was added (50 mL), the batch reactors were monitored for 22 hours,

twice in the first evening, twice the next morning before direct sunlight, and every two hours through the day. Temperature was monitored, but uncontrolled, therefore the batch experiments varied based on sunlight exposure and air temperature, typically from ~ 15 °C at 7 am to a peak of ~ 40 °C at 3 pm.

Table 13: Experimental conditions for the outdoor batch experiments (2.5 L volume)

	HRAP biomass	**Effluent**	**MQ water**	**Pre-treatment**	**TET stock solution [100 mg L^{-1}]**
Active HRAP 100%	2.45 L				50 mL
Dead 100%	2.45 L			121 °C, 20 min	50 mL
Active HRAP 10% in effluent	0.25 L	2.2 L			50 mL
Active HRAP 10% in MQ water	0.25 L		2.2 L		50 mL
Effluent filtrate		2.45 L		Filtering, 0.7 µm	50 mL
MQ water			2.45 L		50 mL

3.4.2.2 Sorption batch tests

The 2.5 L batch reactors described in Section 3.4.2 were used in two batch experiments to measure sorption kinetics and the sorption isotherm for TET with the HRAP biomass. The first of these experiments varied the concentration of TSS, and also tested the effect of dead vs. active biomass. The second of these experiments tested multiple initial concentrations of TET with the active biomass concentration held constant.

For the first experiment, different TSS concentrations of biomass were prepared by dilution of active or autoclaved HRAP biomass with clarified effluent, using dilutions of 100%, 67%, or 33% biomass in effluent. Each batch reactor was filled with 2.45 L of the corresponding biomass, with one control using MQ water. After TET was added (50 mL), the batch reactors were monitored during 16 hours (0, 0.5, 1, 1.5, 2, 3, 4, & 16 h).

For the second sorption batch tests the sorption kinetics and the sorption isotherm for TET were determined by adding different initial TET concentrations. The batch reactors

were filled with 2.25 L of undiluted active biomass and (0.25 – X) L of MQ water, where X is the volume of TET stock solution required to prepare the initial concentrations of 0.2, 0.5, 1, 2, 5, and 10 mg L^{-1}. The batch reactors were incubated at 20 ± 3°C in the dark while TET concentration was monitored over 14 hours (0, 0.5, 1, 4, & 14 h). In addition, 40 mL of biomass samples were taken for sorption extraction analysis at 4 hours and at the final sampling time.

3.4.2.3 pH batch tests

The effect of pH on TET degradation was studied independently, adjusting pH by addition of 0.1 M HCl or 0.1 M NaOH solution to HRAP biomass in batch tests. Four batch reactors were prepared with 2.45 L of active biomass and incubated at 20 ± 3°C under dark conditions. The batch reactors were adjusted to pH 6, 8.5, and 10.5 (Appendix S3.3), leaving one batch reactor with unadjusted pH as a control (typical pH of the control was 6.5-7.0). A 5^{th} batch reactor was filled with MQ water as a second control. The pH values were chosen according to the pKa values for TET (see Section 3.2.1). After the initial pH adjustment, TET stock solution was added (50 mL) and the batch reactors were then monitored over 4 hours in the dark (0, 1, 2, & 4 h). Before each sampling time, the pH was re-adjusted as required to obtain the appropriate pH. Because pH can impact TET analysis, the culture pH was adjusted before each sample was withdrawn to ensure consistent analysis.

3.5 Batch experiments with antibiotic mixtures

Subsequent to the main experimental work studying tetracycline, the batch experimental methods developed for the 2.5 L batch reactors were also used to investigate antibiotic removal with antibiotic mixtures. This work with other antibiotics was performed to make a preliminary assessment of the antibiotic removal mechanisms under conditions relevant to HRAPs, in order to compare and contrast the findings with the main experimental work on TET.

Two different mixtures of three antibiotics each were used: 1) "STS mix" = Sulfanilamide, Tetracycline, and Sulfamethoxazole; 2) "SCC mix" = Sulfanilamide, Ciprofloxacin, and Chloramphenicol. TET was used in one of the antibiotic mixtures, to assess if the presence of other antibiotics affected the TET removal mechanisms. Sulfanilamide was used in both the "STS mix" and the "SCC mix", to provide a common point for comparing the two mixtures.

In the below sub-sections, each antibiotic is briefly described,[a] and then the batch experiments performed with the two antibiotic mixtures is summarized.

3.5.1 Background information about sulfanilamide, sulfamethoxazole, ciprofloxacin, and chloramphenicol

The selected antibiotics were chosen for investigation as there was a wide variety of structures between the different antibiotics (Figure 6), and all the antibiotics could be detected using same the HPLC[b] method used to detect TET (Section 3.6).

Sulfanilamide (SFL) and sulfamethoxazole (SMX) are both antibiotics in the class of sulfanilamides. SMX is a derivative of sulfanilamide, and has a pKa at 5.6 (Chen et al.,

[a] except for TET, which was described in Section 3.2.1
[b] High performance liquid chromatography.

2011) associated with the secondary amine between the two rings (Figure 6 B). SFL and SMX are commonly removed by sorption and biodegradation in activated sludge, although conjugation may prevent full removal (Casas et al., 2015; Ingerslev and Halling-Sorensen, 2000; Kovalova et al., 2012; Yang et al., 2012). Photodegradation of both SFL and SMX has been reported in purified water under simulated sunlight (García-Galán et al., 2008; Gmurek et al., 2015), and in wetlands, SMX removal was enhanced under anaerobic conditions (Rühmland et al., 2015).

Ciprofloxacin (CPX), a fluoroquinolone antibiotic, has two pKa's at 6.2 and 8.8 (Chen et al., 2011) associated with the carboxylic acid and the cyclo-secondary amine, respectively (Figure 6 C). CPX is resistant to biodegradation, but is commonly removed by sorption in activated sludge, and can photodegrade in surface waters (Babić et al., 2013; Li and Zhang, 2010; Porras et al., 2015; Wu et al., 2009).

Chloramphenicol (CMP), has a rather unique structure among the antibiotics tested, with two chlorines and an aromatic nitrite group (Figure 6 D).[a] CMP has variable removal in activated sludge treatment, and removal is attributed to degradation rather than sorption (Kasprzyk-Hordern et al., 2009; Zhou et al., 2013a). In one case, 100% removal of CMP has been reported during WWT with a trickling filter (Kasprzyk-Hordern et al., 2009). CMP can also photodegrade under UV treatment (Zuorro et al., 2014), so there is potential for photodegradation of CMP by sunlight. The physical and chemical properties of the antibiotics used in this thesis are reported in Table 14, Section 3.6.

[a] pKa values have not been listed here, as no consistent reports of the pKa's for CMP could be found.

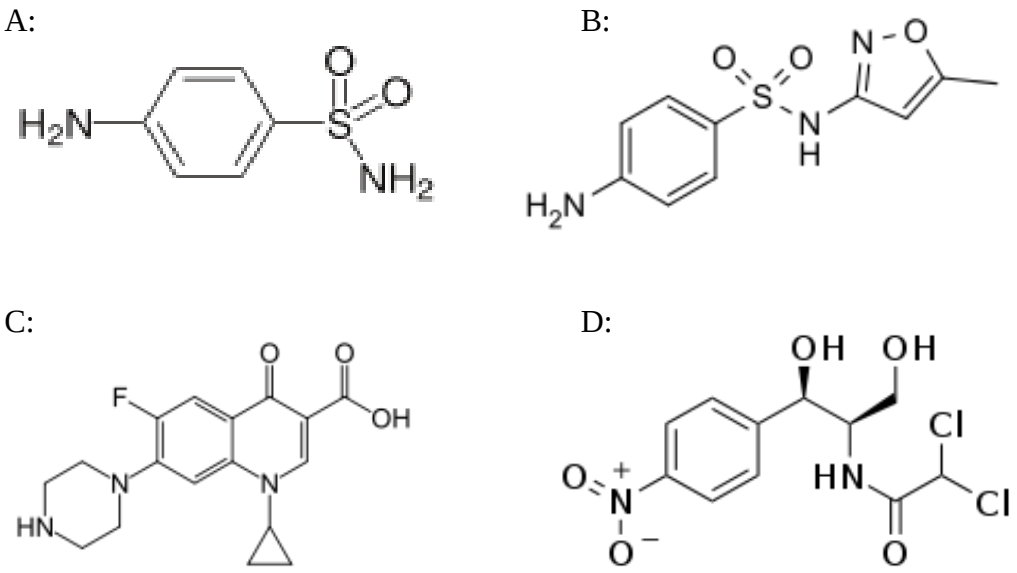

Figure 6: Structure of antibiotics; A: sulfanilamide, B: sulfamethoxazole, C: ciprofloxacin, and D: chloramphenicol

3.5.2 Experiments with the antibiotic mixtures

For each of the two antibiotic mixtures, a stock solution was prepared with the three antibiotics together in MQ water (100 mg of each antibiotic L^{-1}). To dissolve the ciprofloxacin, 0.2% formic acid was added to the "SCC mix" stock solution, and thoroughly mixed to dissolve the antibiotics before raising the solution to neutral pH with dropwise addition of 0.1 M NaOH and making the solution up to a set volume with MQ water.

For each mixture of antibiotics an outdoor full-day batch test was conducted (Section 3.4.2), with relevant results shown in the thesis. pH batch tests (Section 3.4.2.3) were also conducted for the antibiotic mixtures, but the results are not reported in this thesis, as some of the findings were uncertain, and did not add to the discussion.

3.6 Analytical methods for antibiotics

Stock solutions of antibiotics were prepared from analytical grade powders (Sigma Aldrich) in MQ water. Stock solutions were prepared fresh weekly. The physical and chemical properties of the antibiotics used in this thesis are shown in Table 14.

Table 14: Physical and chemical properties of antibiotics used in this study.[a]

	Chloramphenicol	**Ciprofloxacin**	**Sulfamethoxazole**	**Sulfanilamide**	**Tetracycline**
Aqueous Solubility at 25 °C [mg L^{-1}]	2,500	30,000	610	7,500	231
pKa's	n.a.[b]	6.2 & 8.8	1.7 & 5.6	10.58	3.3, 7.8, & 9.6
Mw	323.1	331.4	253.3	172.2	444.4
Henry's law constant at 25 °C [atm-m3/mole]	2.29×10^{-18}	5.09×10^{-19}	6.42×10^{-13}	1.50×10^{-10}	4.66×10^{-24}
log Kow	1.14	0.28	0.89	-0.62	-1.3
Melting point [°C]	150.5	n.a.	167	165.5	172.5
Vapour pressure at 25 °C [mm Hg]	1.73×10^{-12}	1.65×10^{-12}	6.93×10^{-8}	7.30×10^{-6}	3.09×10^{-23}

[a] Properties sourced from ChemIDplus (https://chem.nlm.nih.gov/chemidplus/rn/56-75-7) Retrieved Nov 2016, with some of the pKa values from Chen et al. (2011) and Qiand and Adams (2004).
[b] consistent pKa data was not found for chloramphenicol

A number of HPLC procedures were tested, before selecting an adapted version of an HPLC protocol for antibiotic detection developed by Phenomenex. The HPLC procedure utilised a Kinetex Core-shell C-18 150 mm × 4.6 mm column with 2 µm packing, 100 Å pores, eluted with a 1.5 mL min^{-1} or 1 mL min^{-1} flow with gradient between Solvent A (acetonitrile with 0.1% formic acid) and Solvent B (water with 0.1% formic acid). The flow was reduced to 1 mL min^{-1} due to issues with high pressure. Gradient of Solvent A for 1.5 mL min^{-1} flow started at 3%, increasing to 55% at 3 min, 95% from 3.5 min – 4.5 min, and returned to 3% at 4.6 min (total run time 6 min). For the 1 mL min^{-1} flow, the times were extended proportionally: Solvent A started at 3%, increasing to 55% at 4.5 min, 95% from 4.7-6.9 min, decreasing to 3% at 7 min (total run time 9 min). The column was maintained at 25°C. Standard injection volume was 50 µL, and calibrations were carried out regularly using freshly prepared 0.2 mg L^{-1} and 2 mg L^{-1} stock solutions with varied injection volumes between 1-100 µL. The quantification limit was 0.02 mg L^{-1}, with a strong linear relationship. For a short period (Jan-Feb 2015), a Merck Chromolith® RP-18e 100 × 4.6 mm column was used, with the 1.5 mL min^{-1} flowrate, as it had a lower pressure drop over the column. Quantification limits and retention times were similar to the Phenomenex column, and use of the Merck column was stopped due to an issue with the guard column. Both columns were used with the supplier's recommended C-18 guard cartridges, and cartridges were replaced approximately every 2 months of regular use, or when pressure increases indicated a blockage in the guard cartridge. In NZ, a Dionex Ultimate 3000 HPLC system was used with UV-vis diode-array-detection, and peak areas were analysed with Chromeleon 7 software. In Spain, a Waters e2695 HPLC system was used with a 2998 PDA detector, and peak areas were analysed using Empower 3

software. The reproducibility of HPLC analysis was experimentally estimated to 3% by multiple injection of the same sample.

TET and all other antibiotics tested for this thesis were recorded at their maximum absorption spectra using a diode array detector. TET and 4-epi-tetracycline (4epiTET) were detected at 360 nm, ciprofloxacin (CPX) and chloramphenicol (CMP) at 278 nm, sulfanilamide (SFL) and sulfamethoxazole (SMX) at 270 nm, and phenol at 272 nm. Retention time of TET was 3.5-3.6 min with a 1.5 mL min^{-1} flow and 4.6-4.7 min with a 1 mL min^{-1} flow. Other antibiotics and phenol were all analysed with a 1 mL min^{-1} flow, and were detected at the following retention times: CPX ≈ 4.3 min, CMP ≈ 5.7 min, SFL ≈ 3.0 min, SFX ≈ 5.6 min, phenol ≈ 5.5 min.

For TET quantification in this thesis, the concentration values for TET quoted are a summation of 4epiTET and TET unless specified otherwise. 4epiTET is an isomer of TET that exists in equilibrium with TET in water, has a similar absorption spectrum near 360 nm, and still has antibiotic activity (McCormick et al. 1957).

To quantify sorption of antibiotics to biomass, an extraction procedure was adapted from the methods reviewed by Anderson et al. (2005) for TET extraction from meat tissue. A range of solvents and extraction procedures were tested on algal biomass, resulting in the final procedure. Each 50 mL sample of biomass of known dry weight was centrifuged at 20,000 G for 5 min in a tared centrifuge tube. The supernatant was removed, the pellet was weighed to calculate the volume of water remaining with the biomass, and 10 mL solvent was added to the pellet (1% formic acid, 25% ACN, 75% H_2O). The solvent/biomass sample in the centrifuge tube was placed horizontally on a shaker platform and mixed overnight (12-19 hours) at 350 rpm. The TET concentration of the second supernatant sample is then quantified by HPLC. In NZ, supernatant was

clarified by centrifugation, and in Spain 0.2 µm nylon filters were used to filter the sample. The amount of TET originally sorbed to the biomass was calculated by mass balance.

Solid phase extraction (SPE) procedure for the 180 L pilot HRAP TET analysis (Section 3.3.3) was adapted from Yang et al. (2005). Samples were immediately filtered with a combined glass filter (0.7 µm) & cellulose-acetate Millipore (0.45µm) filter. The required sample volume (50 mL for WW samples, 100 mL for HRAP or effluent samples) were measured and 1 mL of 5% Na_2EDTA added. If SPE was not immediately performed, samples were stored in the freezer (- 4°C) for up to a week (no significant degradation was observed during storage in the freezer), and defrosted in room temperature water for further analysis. SPE cartridges (Oasis HLB Plus Short Cartridge, 225 mg sorbent per cartridge, 60 µm particle size) were prepared by 3 consecutive washed: 1) 3 mL MeOH; 2) 3 mL 0.5 N HCl; 3) 3 mL MQ water. 20mL of citric acid was then added to the 50 or 100 mL sample. 0.5N HCL was added dropwise until pH was <3. The acidified sample was dripped through the prepared SPE cartridge at 5 mL min^{-1}, followed by a 3 mL MQ water rinse. Excess water was expelled by forcing air through the cartridge with a syringe. Cartridges were then extracted using 2 mL MeOH (early samples extracted with 5 mL, but breakthrough tests showed that >99% of the detected TET was in the first 2 mLs). The eluent was stored in HPLC vials in a -4°C freezer for no more than 2 weeks before analysis. Samples were defrosted and analysed by HPLC as described above, but with a 25 µL standard injection volume, as calibration curves of TET in methanol showed non-linear behaviour above 30 µL injection volume.

Table 15: Quality control parameters of analytical methods

Sample preparation	LOD[a]	LOQ[b]	Recovery[c]	Average analysis error[d]
	µg L^{-1}	µg L^{-1}	% mean ± st. dev. (n)	± % difference in paired replicates (n)
Filtration for aqueous TET conc. (NZ)	5	20	91 ± 6 (9)	3 (12) [e]
Filtration for aqueous TET conc. (Spain)	5	20	95 ± 20 (9)	3 (15)
Filtration and SPE for aqueous TET conc. (Spain)	0.1	2	91 ± 5 (4)	14 (5)
Solvent extraction of sorbed TET from biomass (NZ)[f]	5	20	84 ± 9 (19)	20 (16)
Solvent extraction of sorbed TET from biomass (Spain)	5	20	94 ± 7 (6)	4 (8)
Filtration for aqueous SMX conc. (Spain)	5	20	109 ± 11 (5)	n.a.
Filtration for aqueous CPX conc. (Spain)	5	20	91 ± 4 (3)	n.a.
Filtration for aqueous SFL conc. (Spain)	5	20	100 ±2 (4)	n.a.
Filtration for aqueous CMP conc. (Spain)	5	20	96 ± 2 (4)	n.a.

[a] Limit of detection.
[b] Limit of quantification.
[c] The recovery of antibiotics from water is reported for control tests in MQ water across separate experiments. For sorbed TET recovery, statistics are based on quality control tests conducted with known TET concentrations in the extraction solvent incubated with centrifuged HRAP biomass pellets overnight.
[d] When duplicate samples were withdrawn from batch assays or HRAPs, these samples were filtered and analysed in parallel under identical conditions. The average relative difference (%) calculated based on all duplicates carried out (for a specific set of experiment) is henceforth referred to analysis error and displayed above.
[e] The analysis error was 11% (n = 22) for the 900 L pilot scale HRAP. This higher variation between duplicate analysis of samples from the HRAP was due to the larger scale of the HRAP.
[f] The LOD and LOQ of solvent extraction are based on TET concentrations in the extraction solvent. The LOD and LOQ values per g biomass depend on the TSS concentrations and solvent volumes used, so these values were highly variable and not summarised in this table.

3.7 Other analytical methods

Standard methods for the analysis of WW and HRAP effluents were used (Clesceri et al., 1998). TSS and VSS were measured with 47 mm glass fibre filters (pore size: 1.2 µm, NZ; 0.7 µm, Spain), dried at 100-105°C (TSS), and ashed at 550°C (VSS). Manometric BOD tests were used in NZ for WW samples. COD analysis in NZ used the colourmetric method with pre-prepared reagent tubes (ThermoScientific 0-150 mg/L

and 0-1500 mg/L COD tubes measured with a Thermoscientific Orion Aquafast AQ3700 Colorimetry Meter). COD analysis in Spain was analysed by titration with 2.5 mL samples.

Total organic carbon (TOC) and total nitrogen (TN) analysis was measured using a Shimadzu TOC analyser (Modules TOC-L and TNM-L in NZ; TOC-VWP and TNM-1 in Spain). Filtered TOC and TN samples were prepared from the filtrate of glass fibre filters (pore size: 1.2 µm, NZ; 0.7 µm, Spain).

Dissolved anions were analysed by ion chromatography with ion conductivity detection after filtration with 0.2 µm syringe filters (NZ: mixed cellulose esters; Spain: nylon). In NZ, a Dionex ICS-2000 ion chromatograph was used, with a mobile phase of KOH gradient in MQ water and a Dionex IonPac^{TM} AS18 4x250mm column and an IC-Pak Anion Guard-Pak (Waters), 25 µL injection. Anions (NO_2^-, NO_3^-, PO_4^{-3}) were analyzed by ion chromatography as described in literature (Posadas et al., 2015b). In Spain, N-NH_4^+ was measured using an Orion DualStar ammonia electrode (Thermo Scientific, The Netherlands).

UV and visible light absorbance measurements for algal cultures were measured in 1 cm cuvettes in a spectrophotometer (NZ: Thermo electron corporation Heλios γ; Spain: Hach DR/4000V, US).

PAR light was measured using a LI-COR quantum Sensor, with either a LI-COR handheld meter (NZ and Spain) or a Watchdog data-logger (NZ). In Spain, light was data-logged in lux using a light meter (PCE-174, Albacete, Spain), and regular measurement of PAR light recorded and lux light recorded at several sunlight intensities were used to create a correlation between the two data sets and convert the data-logged

lux data to PAR units for use in modelling. A hand-held UVA/B light meter (Sper Scientific) was used to measure UV light in NZ.

An actinometry method was used to quantify average light intensity for the lab-scale HRAPs and indoor beaker batch tests using potassium ferric oxalate (method adapted from Hatchard and Parker (1956)). The light spectrum was measured by an Optronics OL756 spectroradiometer (Orlando, FL, USA). Full method and results of the actinometry experiment is reported in Appendix Four.

In NZ, the pH, DO and temperature were measured using an Orion Star A326 multi-meter with data-logging capability. The pH probe was a ROSS Ultra pH/ATC Triode® gel-filled electrode, and the DO probe was a ThermoScientific Orion RDO® optical DO sensor. Both the pH probe and DO probe recorded temperature. In Spain, a Consort multi-logger with a 3m Consort DO probe, and Bioblock Scientific 22.5 cm pH probe were used for continuous data-logging of the 180 L HRAP, and for measuring the full-day 2.5 L batch trials. For other pH and DO measurements in Spain, a CyberScan pH 510 meter and a handheld OXI 330i oximeter (WTW, Germany) were used.

In NZ, morphological identification and relative abundance of algae was carried out by Cawthron Institue, Nelson, NZ. Two 100 mL algae samples were sent for analysis by overnight courier, with 1 sample preserved with 2 drops of Lugol solution. In Spain, the morphological identification of microalgae was carried out by microscopic observations (OLYMPUS IX70, USA) on two 1.5 mL samples preserved in 10% formaldehyde or fixation with 5% of Lugol's iodine. In Spain, composition analysis was also carried out on the settled biomass. The harvested biomass in the settler was dried for 24 h at 105 °C in a P-Selecta laboratory stove (SELECTA, Spain). The determination of the C and N

content of the algal-bacterial biomass was performed using a LECO CHNS-932, while phosphorus content analysis was carried out spectrophotometrically after acid digestion in a microwave according to the internal procedure of the Instrumental Technical Laboratory of the University of Valladolid.

Photosynthetic efficiency for the lab-scale HRAPs was calculated by the method described by Alcántara et al. (2015).

4. Results and Discussion

In this section, the results from the experimental work are discussed, grouped into sub-sections by the type of experiment. The results begin with the study of TET fate in lab-scale HRAPs in NZ (Section 4.1), and then the results from the 100 mL batch experiments under artificial light performed in NZ are presented, which were used to distinguish different TET removal mechanisms (Section 4.2). In Section 4.3, the results from the 2.5 L batch experiments performed in Spain are then presented; these experiments were used to test the findings from the 100 mL batch experiments under sunlight and investigate some TET removal mechanisms further. Then in Section 4.4, the results from TET removal experiments in the two pilot HRAPs performed in NZ and Spain are presented; the experiments in the pilot HRAPs were used to test the findings from the lab-scale HRAPs experiments and some of the findings from the batch experiments. In order to predict TET removal in HRAP with different designs and operations, a kinetic model for predicting the TET removal is developed and discussed in Section 4.5. This model is then used to help compare the TET removal results between the batch experiments and the research in the HRAPs in Section 4.6. Finally, some preliminary trials investigating the removal of other antibiotics are presented in Section 4.7, to compare and contrast the results from the removal of other antibiotics with the TET removal in HRAP.

4.1 Indoor Lab HRAP

In this section, results are presented from the operation of indoor lab-scale HRAPs over a period of 7 months in order to assess how TET removal was affected by different periods of light exposure at two different HRTs. The HRAPs were described in Section 3.3.1.

Five stages of HRAP operation were tested:

- 7 day HRT with 12 hours light/24 hours
- 4 days HRT with 12 hours light/24 hours
- 7 days HRT with 8 hours light/24 hours
- 4 days HRT with 8 hours light/24 hours
- 4 days HRT with 16 hours light/24 hours

The 3 periods of light exposure tested were chosen to represent normal diurnal sunlight hours in spring and autumn (12h /24 h), low sunlight hours in winter (8 h/24 h), and high sunlight hours in summer (16h /24 h). Seven-day HRT was selected for the initial HRT of HRAP operation, based on typical values for HRAP discussed in the literature review (see Table 1, Section 2.2). The HRT was then reduced to 4 days – the lowest typical HRT used for HRAP, to test whether efficient TET removal could be maintained under these conditions.

4.1.1 HRAP performance

Due to the diurnal light supply, temperature varied from 15-23°C diurnally through the day. The associated diurnal variations in pH and oxygen from photosynthetic activity are shown in Table 16. Both HRAPs exhibited consistent total organic carbon (TOC) removal of 74 ± 8% (mean ± st.dev., n=118), and total nitrogen (TN) removal was 52 ± 18 % (mean ± st.dev., n=118). This performance is similar to other HRAPs operated with WW (Park et al., 2013; Posadas et al., 2015a). Photosynthetic efficiency (normally 1.8-2.9%; Appendix Section S1.1.3) was in agreement with reported PE in outdoor algal photo-bioreactors and HRAPs (Béchet et al., 2013; Sutherland et al., 2015). Dominant phototrophs were *Microcystis* spp., *Scenedesmus* sp., *Pediastrum* sp., *Gomponema* sp., and *Pseudanabaenaceae* (see Section 3.7 for identification method).

Table 16: Summary of the environmental conditions and effluent composition under the 5 different operating stages of the lab-scale HRAP. Errors are given as 95% confidence intervals of the data. Some monitored parameters are given as a range since data was not universally stable.
TET supplied in all stages was 2 mg $_{TET}$/ L_WW.

Stage	**Stage I**		**Stage II**		**Stage III**		**Stage IV**		**Stage V**	
Dates of pseudo-steady-state operation	4th Aug to 27th Aug 2014		1st Sep to 15th Sep 2014		10th Oct to 22nd Oct 2014		10th Nov to 29th Nov 2014		5th Dec to 18th Dec 2014	
Pond Name	HRAP A	HRAP B	HRAP A	HRAP B	HRAP A	HRAP B	HRAP A	HRAP B	HRAP A	HRAP B
HRT	7	7	4	4	7	7	4	4	4	4
Light Hours/ 24 h	12	12	12	12	8	8	8	8	16	16
pH AM []	7.5 to 7.9 (14)	7.8 to 7.9 (14)	7.5 to 7.8 (11)	7.2 to 7.6 (11)	7.0 to 7.6 (17)	6.9 to 7.5 (9)	6.5 to 6.9 (8)	6.4 to 7.0 (8)	6.8 to 7.6 (11)	6.8 to 7.5 (11)
pH PM []	9.7 to 10.7 (16)	10.0 to 10.6 (16)	9.4 to 10.8 (10)	9.3 to 10.2 (10)	10.0 to 10.5 (15)	8.9 to 10.2 (8)	6.7 to 8.3 (15)	6.6 to 9.0 (15)	8.1 to 10.4 (9)	8.0 to 10.1 (9)
DO AM [mg L^{-1}]	5.0 to 9.0 (14)	8.4 to 8.9 (14)	8.2 to 9.0 (11)	8.0 to 8.7 (11)	5.2 to 9.3 (17)	8.3 to 9.2 (9)	8.1 to 9.9 (8)	8.4 to 9.1 (8)	7.7 to 8.6 (11)	7.9 to 8.8 (11)
DO PM [mg L^{-1}]	9.1 to 10.6 (16)	9.6 to 10.6 (16)	9.2 to 10.4 (10)	8.8 to 9.6 (10)	8.7 to 10.3 (14)	7.9 to 9.4 (8)	8.0 to 9.2 (15)	7.9 to 9.1 (15)	8.8 to 10.0 (9)	8.7 to 9.9 (9)
TSS [g L^{-1}]	0.47 ± 0.07 (12)	0.41 ± 0.09 (12)	0.29 ± 0.09 (8)	0.20 ± 0.06 (8)	0.30 ± 0.06 (12)	0.27 ± 0.06 (7)	0.10 ± 0.02 (12)	0.14 ± 0.02 (12)	0.29 ± 0.10 (8)	0.32 ± 0.13 (8)
TSS Productivity [kg m^{-2} d^{-1}]	4.42 ± 0.74 (11)	3.89 ± 0.85 (11)	4.47 ± 2.03 (8)	3.27 ± 0.91 (8)	3.05 ± 0.65 (12)	2.82 ± 0.53 (12)	1.76 ± 0.38 (12)	2.22 ± 0.30 (12)	4.93 ± 1.56 (8)	5.42 ± 2.42 (8)
Sorbed TET [mg $gTSS^{-1}$]	0.19 (1)	0.30 (1)	0.50 (1)	0.59 (2)	0.30 (1)	0.39 (1)	1.73 (2)	1.40 (2)	0.95 (2)	0.88 (2)
K_d [L g^{-1}][a]	1.56	3.20	2.11	2.65	1.57	0.87	6.49	5.50	9.28	6.74
Removal of TET by sorption	3.6%	3.5%	6.9%	5.0%	3.2%	7.0%	9.4%	9.8%	9.0%	5.6%
Dissolved TET AM [mg L^{-1}]	0.13 ± 0.02 (13)	0.10 ± 0.02 (13)	0.22 ± 0.05 (8)	0.20 ± 0.02 (8)	0.21 ± 0.05 (10)	0.21 ± 0.08 (6)	0.26 ± 0.03 (10)	0.24 ± 0.04 (10)	0.11 ± 0.08 (9)	0.17 ± 0.16 (9)
Dissolved TET PM [mg L^{-1}]	0.12 ± 0.02 (12)	0.10 ± 0.01 (12)	0.22 ± 0.04 (9)	0.22 ± 0.03 (9)	0.15 ± 0.03 (11)	0.17 ± 0.05 (7)	0.28 ± 0.05 (11)	0.26 ± 0.05 (11)	0.12 ± 0.02 (11)	0.14 ± 0.04 (11)
Overall TET Removal	93.8 ± 0.5%	95 ± 1%	88 ± 1%	89 ± 1%	90 ± 1%	89 ± 2%	88 ± 5%	87 ± 2%	87 ± 2%	95 ± 1%

[a] Sorption partition coefficient $K_d = \frac{[mg_{sorbed.antibiotic}/g_{solid}]}{[mg_{dissolved.antibiotic}/L_{solution}]}$; assuming that sorbed TET concentrations were at equilibrium with the aqueous TET concentrations

4.1.2 TET removal under continuous HRAP operation

TET was consistently removed from the aqueous phase (average removal was between 87-95%; Table 16). Resulting effluent TET concentrations were 0.09-0.27 mg L^{-1} (Figure 7). The greatest variability in effluent TET concentrations was during or following a large increase in grazers[a] in the HRAPs (especially during Stages I & II for HRAP-A, and Stages III & IV for HRAP-B). TET removal by sorption to the biomass was consistently low (3-10% contribution; Table 16), despite a ~4-fold increase in the sorption partition coefficient from Stage III to Stage IV.[b] It is uncertain whether the change was due to the change in the method by which TET influent was added,[c] or due to the change in WW composition that occurred between these two Stages (Table 7).

As discussed in Section 2.5.2, de Godos et al. (2012) reported 70% removal of TET[d] in lab-scale HRAPs, and hypothesized that photodegradation and sorption were the dominant removal mechanisms.[e] During batch tests, de Godos et al. (2012) calculated the sorption partition coefficient to be $K_d = 4.2 \pm 0.4$ L g^{-1}. Based on the reported TET and TSS concentrations, this partition coefficient predicts that there was 30% TET removal by sorption in de Godos et al.'s experiments. The higher contribution of sorption but lower overall removal observed by de Godos et al. (2012) compared to our HRAP is likely a consequence of reduced photodegradation due to lower light supply[f].

[a] Algae grazers observed in the HRAPs were predominantly rotifers – microscopic animals which feed on the algae. The rotifers periodically bloomed, severely disrupting the algal population.
[b] See Table 16 for the definition of the sorption partition coefficient. Sorption is investigated in more detail in Section 4.3.2 Sorption analytical methods are discussed in Section 3.6.
[c] TET was pumped directly to the HRAP in Stages IV and V rather than spiked in the influent WW, see Section 3.3.1
[d] de Godos et al. also used $2mg_{TET}/L$ influent concentration
[e] Although the conditions are different (de Godos et al. used synthetic WW, an algae monoculture, and different lighting, see Section 3.3.1), this study is the best benchmark against which results from the lab-scale HRAP experiments conducted in this thesis can be compared.
[f] See Section 3.3.1– de Godos et al. used 10 W continuous light rather than the 47 W diurnal light used for the lab-HRAPs in this thesis; the ponds design of de Godos et al. also had a lower surface-area-to-volume ratio.

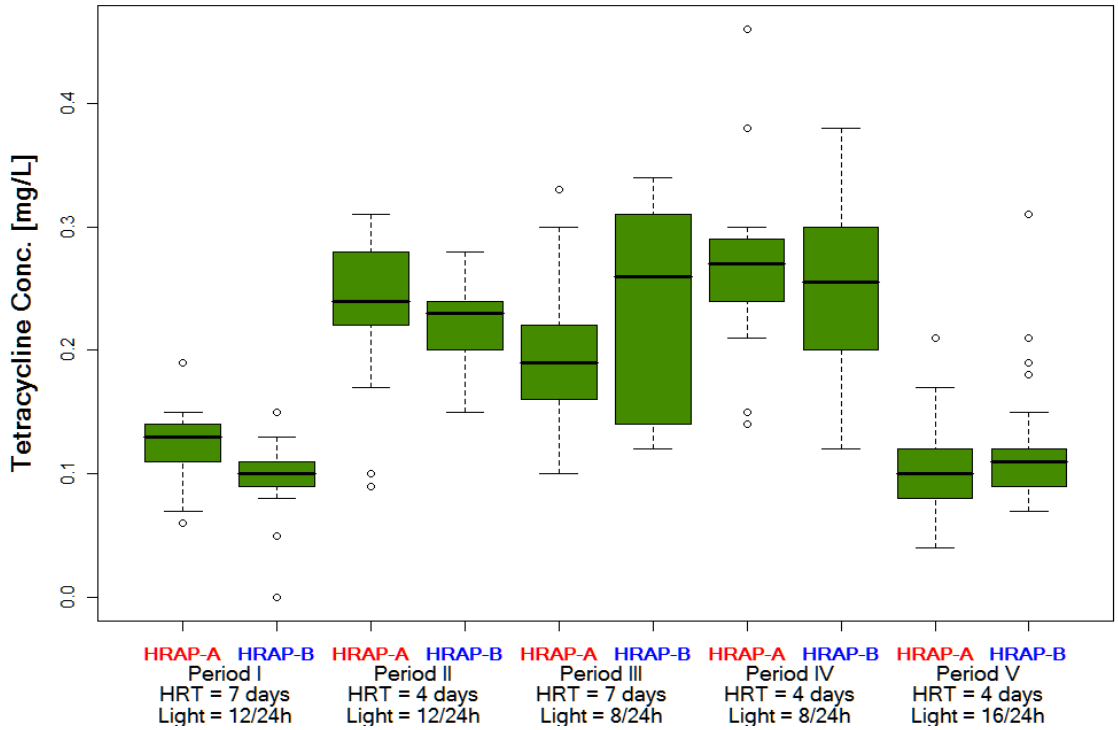

Figure 7: Boxplot of tetracycline removal during lab-HRAP operation. TET supplied in all stages was 2 mg $_{TET}$ L_{WW}^{-1} (see Appendix Section 4.2 for quality control).

To confirm whether photodegradation was significant in our lab-scale HRAPs, the TET concentrations were measured in the morning (dark) and in the evening (light). If photodegradation was the only removal mechanism, then TET concentrations should increase by 0.14 to 0.33 mg L^{-1} during the night, depending on the operational period. However, the TET concentrations did not significantly increase during the night.[a] Temperature or pH variations can affect sorption (see Section 2.3), so sorption extraction tests were also performed for samples collected in the morning and evening of the same day,[b] to test whether diurnal variation in sorption could explain the stable aqueous TET concentrations. However, no significant differences in sorbed TET concentrations were observed, despite temperature and pH variations in the HRAPs.

[a] The p-value (t-test, unequal variances) comparing morning and evening samples was >0.1 for 6 of 10 cases, and 3 of the 4 cases with p-values <0.1 had higher average TET concentrations during the evening samples (see Table 16)
[b] See Appendix One, Section S1.1.4

The reason why aqueous TET concentration remained stable despite diurnal changes in light supply (and therefore photodegradation rates) was not determined for experiments using this lab-scale HRAP.

Despite the lack of significant difference between morning and evening aqueous TET concentrations, decreased TET effluent concentrations were observed in operational stages with increased light supply (Figure 7). This trend is seen clearly in Table 17, where the calculated first-order TET removal kinetic constants[a] change proportionally with the increase or decrease in the light supply. HRAP productivity follows the same trend, as productivity is dependent upon light supply. No clear trends between TET kinetic removal rates and other monitored variables (e.g. TSS, TOC, pH, temp, nutrients; Table 16) were observed. However, between Stage II and Stage III, a correlation was noticed between reduced TET removal and increased inorganic carbon concentration. This correlation occurred after a treatment to remove algal grazers, and is discussed in Section 4.1.3.

[a] Batch experiments (Section 4.2) showed that removal was best explained by first-order kinetic rates. The first-order degradation constant (k_1, d^{-1}) was calculated from the average TET concentrations (C, mg L^{-1}) in each operational period, assuming steady-state operation, where V is the volume of the HRAP (L), and Q is the flowrate (L d^{-1}).:

$k_1 * C * V = (C_{in} - C) * Q$; therefore $k_1 = \frac{C_{in}-C}{C*HRT}$ where $HRT = \frac{V}{Q}$

Table 17: Trends observed between light-hours, productivity, and first-order kinetic rates (k_1) for the continuous degradation of TET in the two 7 L HRAPs (A and B) during the 5 operating stages.

	Stage I, 4th Aug to 27th Aug		**Stage II, 1st Sep to 15th Sep**		**Stage III, 10th Oct to 22nd Oct**		**Stage IV, 10th Nov to 29th Nov**		**Stage V, 5th Dec to 18th Dec**	
HRAP	A	B	A	B	A	B	A	B	A	B
HRT	7	7	4	4	7	7	4	4	4	4
TET [mg L^{-1}]	0.13	0.09	0.24	0.22	0.19	0.23	0.27	0.25	0.10	0.12
k_1 [d^{-1}][a]	2.2 ± 0.2	2.9 ± 0.4	1.9 ± 0.1	2.0 ± 0.1	1.3 ± 0.1	1.1 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	4.4 ± 1.2	3.3 ± 1.5
Light Hours	12	12	12	12	8	8	8	8	16	16
TSS Productivity [kg m^{-2} d^{-1}]	4.4	3.9	4.5	3.3	3.1	2.8	1.8	2.2	4.9	5.4

[a] As the first order rates (k) displayed above were calculated based on the HRT, influent TET concentration, and average effluent TET concentration (see Table 16), the error associated with the rates were calculated based on the percentage error of the average TET effluent concentration (i.e. the errors associated with the influent concentration and the HRT were assumed to be negligible).

Overall, this study of TET removal in lab-scale HRAPs compares favourably with activated sludge treatments. The consistently high (>85%) removal observed is similar to reported TET removal for activated sludge (60-100% removal (Michael et al., 2013), and 32-85% (Batt et al., 2007)); however TET sorption is the dominant removal mechanism in activated sludge (Kim et al., 2005; Li and Zhang, 2010). Because the removal of TET by sorption in these lab-scale HRAPs was low (<10%; Table 16), risks associated with the disposal or reuse of biomass from the HRAPs may be reduced, depending on the end-use of the sludge. TET removal in the lab-scale HRAPs by degradation therefore remains at least 75%, since sorption only transfers TET from the aqueous phase to the biomass solids. The mechanism(s) of this TET degradation are not evident from these HRAP experiments, as increased rates of TET removal were correlated with both light supply and biomass productivity. Batch tests (Section 4.2) were therefore used to distinguish the mechanisms.

4.1.3 Disruption to tetracycline removal during treatment to remove algal grazers

This section is a digression from discussion of the steady-state TET removal in HRAPs, to relate an interesting correlation between reduced TET removal and increased inorganic carbon in HRAP-B. This correlation was observed subsequent to a treatment applied to the HRAPs to kill algal grazers, between the pseudo-steady-state operating stages II and III, and may be related to the indirect photodegradation of TET.

Treatment of the HRAPs to kill algal grazers (particularly rotifers) was performed in order to reduce the variability in TET removal caused by problems with grazers. The treatment required the addition of NH_4^+ and maintenance of the HRAPs at high pH (>9, preferably 10) for 24 hours. HRAP A maintained this pH with natural algal activity, but HRAP B required the addition of NaOH, because it was more severely affected by the grazers. The TET concentration in HRAP-A remained steady, but the TET concentration in HRAP B increased immediately after the treatment, slowly returning to the previous steady-state (Figure 8). The most likely cause is that NaOH addition increased the concentration of carbonate in HRAP-B via carbonate equilibration with the CO_2 in the air, and this change was recorded in the inorganic carbon measurements (Figure 8). Carbonate can quench hydroxyl radicals (Vione et al. 2006), which suggests that the addition of NaOH inhibited the indirect photodegradation of TET by the carbonate quenching hydroxyl radicals.[a] Correlations between TET concentration and other variables (e.g. pH [0.71],[b] temperature [0.36] and TSS concentration [0.62]) were also investigated, but the strongest correlation observed was with the inorganic carbon concentrations (0.95).

[a] Due to other experimental priorities, and because the correlation between TET degradation and IC concentrations was discovered after the lab HRAPs were decommissioned, the concentrations of hydroxyl radicals were not measured, and their effect was not explored with other hydroxyl radical scavengers

[b] See Section 4.3.3 for results of further investigations into the effect of pH on TET removal.

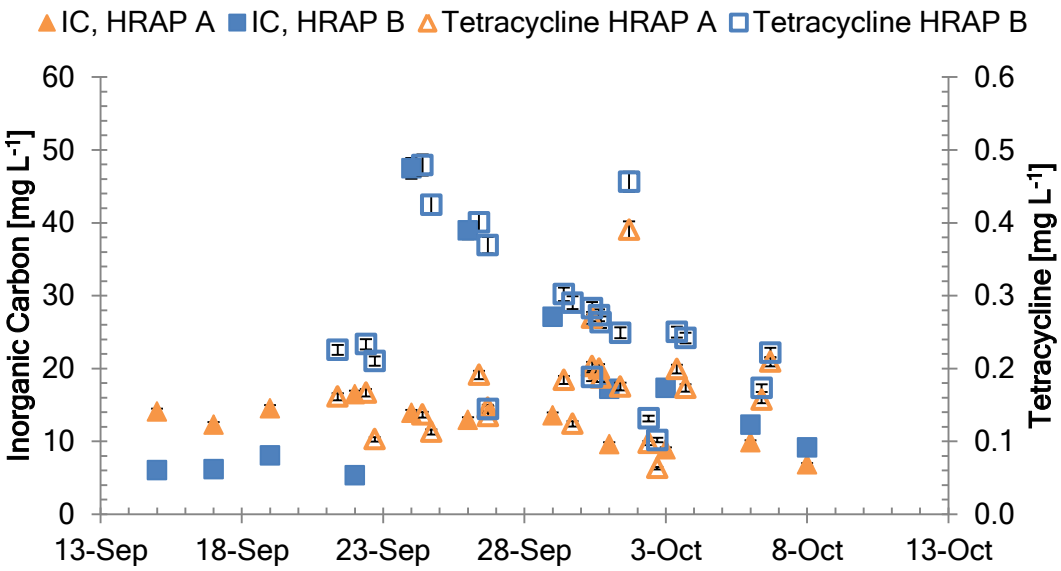

Figure 8: Correlation observed between an increase in inorganic carbon (IC) for HRAP B after rotifer treatment, and the corresponding reduction in TET removal. Rotifer treatment (22nd Sep 2014) involved addition of urea and continuous 36 hours light to maintain pH above 9. HRAP-B required addition of NaOH to increase pH above 9. HRAP-A did not require NaOH during the rotifer treatment, and shows no significant increase in TET during this stage. HRT was 7 days. Light supply changed from 12 hours before the rotifer treatment to 8 hours after the rotifer treatment. Error bars show typical analysis error (see Table 15).

4.2 Batch Experiments - 100 mL

The indoor laboratory-scale batches that are discussed in this section were conducted in parallel with the continuous indoor HRAP experimentation discussed in the previous section (Section 4.1). The purpose for these experiments was to quantify the relative significance of the main removal mechanisms identified in the literature review (Section 2): hydrolysis, sorption, biodegradation, direct photolysis, and indirect photodegradation. The conditions were therefore designed to distinguish the effects of each mechanism.

At least one batch trial was performed during each operational stage of the continuous lab-scale HRAP, discussed in Section 4.1. An example of these batch tests is shown in Figure 9, with TET removal kinetic rates for all the batch tests summarised in Table 18.[a] Because the HRAP operation changed between each of the batch tests shown in Table 18, the characteristics of the algal-bacterial biomass and effluent sourced from the lab-scale HRAP for each batch test varied (see Table 16 for the average effluent characteristics in each operational stage). This variation in the characteristics of the algal-bacterial biomass and effluent used for the batch tests likely explains the variation in the pseudo-first order TET removal kinetics recorded in these assays (see Table 18).

Overall, photodegradation was much greater than any of the other possible mechanisms – this can be clearly seen in Figure 9, with ~90% TET removal in the presence of biomass under light conditions (2 mg L^{-1} initial TET concentration), and is discussed

[a] Full data for the batch tests in Table 18 is provided in Appendix S2.

further below. Pseudo-first order kinetics provided the best fit (greater R^2) for the TET removal rates compared with zero-order rates (Appendix S2) and second-order rates.[a]

Table 18: Pseudo-first-order TET removal constants (k_1) for batch tests with k_1 [d^{-1}] ± st.error (R^2). Regression was calculated based the combined data from HRAP-A & HRAP-B

HRAP operating conditions	Light			Dark		
	Active Biomass	Autoclaved Biomass	MQ water (Abiotic)	Active Biomass	Autoclaved Biomass	MQ water (Abiotic)
Stage I: 7 day HRT 12/24h light	2.5 ± 0.4 (0.86)	3.2 ± 0.3 (0.94)	0.14 ± 0.01 (0.98)	0.16 ± 0.03 (0.82)	0.19 ± 0.03 (0.83)	0.00 ± 0.00 (0.22)
Stage II: 4 day HRT 12/24h light	2.6 ± 0.2 (0.97)	3.5 ± 0.1 (0.99)	0.14 ± 0.02 (0.95)	0.06 ± 0.01 (0.79)	0.06 ± 0.02 (0.59)	0.00 ± 0.02 (0.02)
Stage III: 7 day HRT 8/24h light	1.4 ± 0.1 (0.90)	1.3 ± 0.2 (0.86)	0.14 ± 0.02 (0.90)	0.11 ± 0.03 (0.67)	0.19 ± 0.04 (0.65)	0.04 ± 0.01 (0.83)
Stage IV: 4 day HRT 8/24h light	0.8 ± 0.1 (0.88)	1.5 ± 0.1 (0.95)	0.32 ± 0.11 (0.80)	0.08 ± 0.05 (0.28)	0.07 ± 0.05 (0.30)	0.08 ± 0.06 (0.46)
Stage V: 4 day HRT 16/24h light	2.5 ± 0.3 (0.92)	2.2 ± 0.2 (0.93)	0.20 ± 0.04 (0.90)	0.30 ± 0.04 (0.88)	0.30 ± 0.05 (0.81)	0.01 ± 0.05 (0.02)

[a] Pseudo-first order kinetic rates are commonly reported in antibiotics photodegradation studies (see Section 2.5.1). Second-order kinetic rates are unlikely at low pollutant concentrations, since second-order dependence on TET concentration would require the interaction of two TET molecules during degradation.

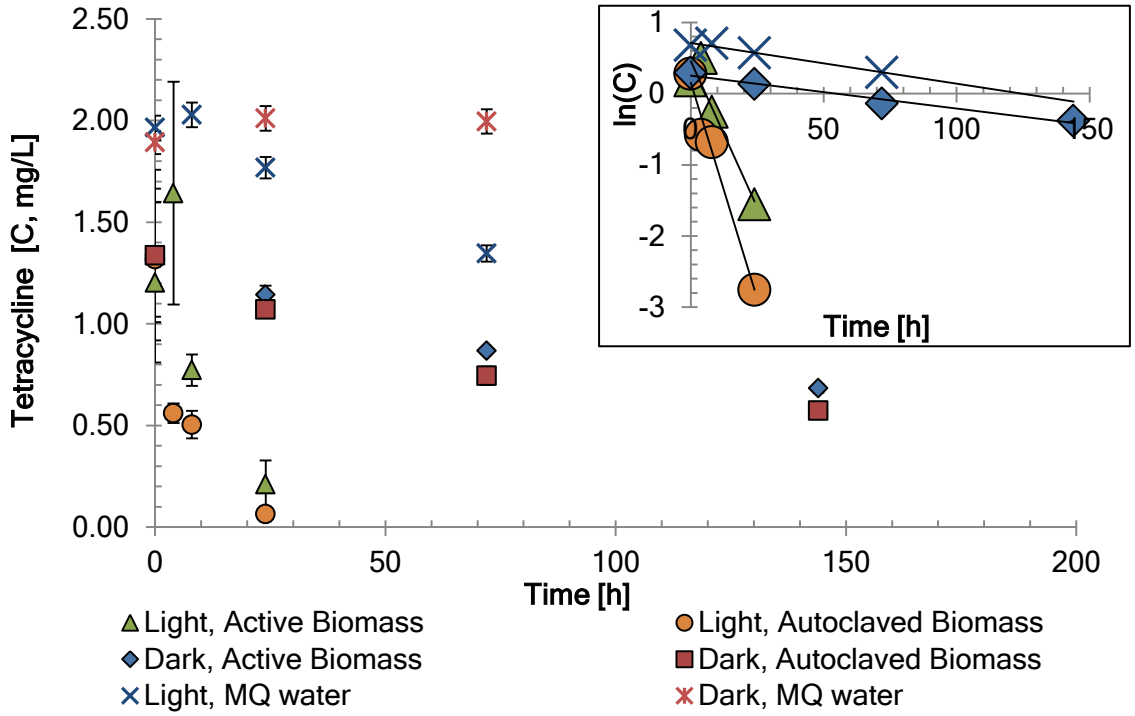

Figure 9: Batch degradation of tetracycline solutions under light or dark conditions. This batch was performed 26th August 2014, when the HRAPs were under 7 d HRT and 12 hour daily illumination. Data for the two HRAPs (A & B) were averaged: the error bars associated with samples containing biomass represent the experimental data range whereas the error bars associated with samples from MQ water controls represent the analytical error (see Table 15). Inset: regression of pseudo-first order kinetic rates for four of the samples.

In these batch tests (Table 18, e.g. Figure 9), consistent trends were observed:

- Hydrolysis of TET was negligible: In the MQ water controls in the dark, only hydrolysis and sorption to the glassware could occur –with no presence of biomass, biodegradation and sorption were prevented, and with no light exposure photodegradation was prevented. Where seen, removal rates for the MQ water control in the dark were generally within the bounds of experimental error, therefore hydrolysis and sorption to glassware was negligible. The

negligible hydrolysis is in agreement with de Godos et al (2012), who also reported insignificant removal of detectable TETs in his abiotic synthetic WW dark controls during batch experiments. Kang et al (2012) reported first-order hydrolysis rates of 0.072 d^{-1} at pH 7, but this included reversible epimerisation (e.g. to 4-epi-TET). Conversion to 4-epiTET is not being considered in this thesis as a full removal mechanism because the equilibrium is easily reversible, and 4-epiTET still has antibiotic activity (as discussed in Section 3.5)

- Sorption of TET was observed in the presence of active or autoclaved biomass under dark conditions: Since hydrolysis was excluded as negligible, and no biodegradation could occur by the autoclaved biomass, sorption is the best explanation for the TET removal in the presence of autoclaved biomass in the dark.
- Biodegradation of TET was negligible: removal rates were almost identical in the presence of active or autoclaved biomass under dark conditions, and the autoclaved biomass should have no capacity for biodegradation. This finding of negligible biodegradation is in agreement with results by de Godos et al. (2012), who studied TET removal in the presence of HRAP biomass dominated by *Chlorella vulgaris* in synthetic WW. However, this finding is in contrast to the results of Dzomba et al. (2015), who reported slow biodegradation of TET by four algal species (see Section 2.4.2). The small differences observed between results in the presence of active or autoclaved biomass (Table 18; Stage I and Stage III) could be due to changes in sorption isotherms from autoclaving the biomass (denaturing proteins, disrupting cell membranes: Prabakaran &

Ravindran 2011), or from the difference in pH between different biomass conditions[a].

- Direct photolysis of TET was minimal: having excluded hydrolysis as negligible, the degradation of TET in the MQ water controls under light should only occur by direct photolysis – there were no other organic materials present that could be involved in indirect photodegradation mechanisms[b]. Garcia-Rodriguez et al. (2013) reported a degradation of 0.10 d^{-1} for TET in MQ water which is in a similar range, despite differences in light supply[c].
- Indirect photodegradation was the dominant TET removal mechanism in these experiments: because the mechanism of indirect photodegradation requires the presence of dissolved organics to generate reactive oxygen species (Section 2.5.1), indirect photodegradation should not be possible in the MQ water controls with no dissolved organics, but should be possible in the presence of the active and autoclaved biomass.[d] Indeed, the TET removal rates in the presence of the active and autoclaved biomass were generally 10-fold to 20-fold higher compared with the photolysis observed in the MQ water controls under light conditions, [e] which means that indirect photodegradation is the dominant TET removal mechanism under these batch conditions. Despite higher final pH in

[a] For example, for Stage I autoclaved biomass in the dark had a pH of 8.3; active biomass in the dark had a pH of 7.9.
[b] Indirect photodegradation of TET is still technically possible, if the TET molecule itself generates the reactive oxygen species required for indirect photodegradation (Chen et al., 2008; Niu et al., 2013; Werner et al., 2006). However, we could not distinguish this mechanism from direct photolysis in these batch tests, and whether the TET photodegradation in MQ water occurs by 'true' direct photolysis or self-sensitized generation of reactive oxygen species does not change the overall findings.
[c] Garcia-Rodriguez et al. used cool daylight fluorescent tubes light intensity of 15 µmol m^{-2} s^{-1}, and 2.5 L reactors, but the geometry of their 2.5 L reactor was not clearly described, which prevents a comparison of their kinetic rates with the rates calculated for the batch experiments in this section based on the surface area and light intensity differences between the two batch experimental methods.
[d] As noted previously, indirect photodegradation should ideally be confirmed using quenchers or direct measurement of reactive species, but the conclusion that indirect photodegradation is the main mechanism in the presence of dissolved organics is consistent with literature investigating indirect photodegradation (see Section 2.5).
[e] In all cases, this difference was statistically significant ($p < 0.01$)

presence of active biomass under light conditions compared to the presence of autoclaved biomass (except Stage IV[a]), no difference in removal rates were observed between the biomass conditions. The range of TET removal noted for all biomass conditions under light across all the regular batch tests was k = 0.82 – 3.00 d^{-1} which is similar to values by de Godos et al. (2012), who reported first-order kinetic rates at 0.91 – 2.18 d^{-1} in closed glass flasks, despite differences in light supply.[b]

In addition to the regular batch tests discussed above, a batch test was conducted to investigate the effects of biomass concentration upon TET removal in the light (Figure 10), to consider the possibilities that HRAP biomass shading might reduce photodegradation at high TSS concentrations.[c] To obtain the different TSS concentrations, biomass from the lab-scale HRAPs was centrifuged and re-suspended in WW (50% strength, to provide carbon and nutrients. See Table 7 Section 3.3.1 for WW composition) [d]. In Figure 10, the results from the control in the dark with 50% WW but no added algal biomass incubated in the dark confirmed that TET sorption to the WW solids was observed within 5-8 hours. This control in the dark also confirmed that no biodegradation by the bacteria introduced with the WW was observed, as TET concentrations were stable between 8-24 h. Photodegradation under these conditions

[a] During the batch test conducted for Period IV 1st Dec 2014, there was little increase in pH during the batch test. The biomass was collected from the HRAP during operation with 4 days HRT and 8 hours light, which led to wash-out of a lot of biomass and low productivity, which may explain the low pH change, if the biomass was stressed. The fact that this batch test was the only one to show a difference between active and autoclaved biomass conditions in the light suggests that high pH may enhance degradation, but this effect is not observed in the other batch tests because indirect photodegradation in the active biomass sample is usually lower, and then the increased pH masks the difference between the active and autoclaved biomass conditions. However, this is very speculative as the suggestion is based only on the one experiment.

[b] de Godos et al. used lights with lower PAR (10 W m^{-2}), compared with 16 W m^{-2} used in the batch experiments in this section, and the use of closed glass flasks compared with open beakers would also affect the light supply.

[c] Tests were also conducted with filtered HRAP effluent (Appendix S2 - Table 15). There was little difference between the rates of TET removal in filtered versus unfiltered HRAP effluents.

[d] The characteristics of the HRAP cultures used in the other batch tests are shown in Appendix S1.1.2, Table 2.

was not affected by the biomass concentration, as similar removal rates were observed at all TSS concentrations under light (0-0.5 g_{TSS} L^{-1} HRAP biomass; Figure 10). In contrast, de Godos et al. (2012) reported that TET removal rates did depend on TSS concentration, with shading effects noted above 88 mg L^{-1} TSS.

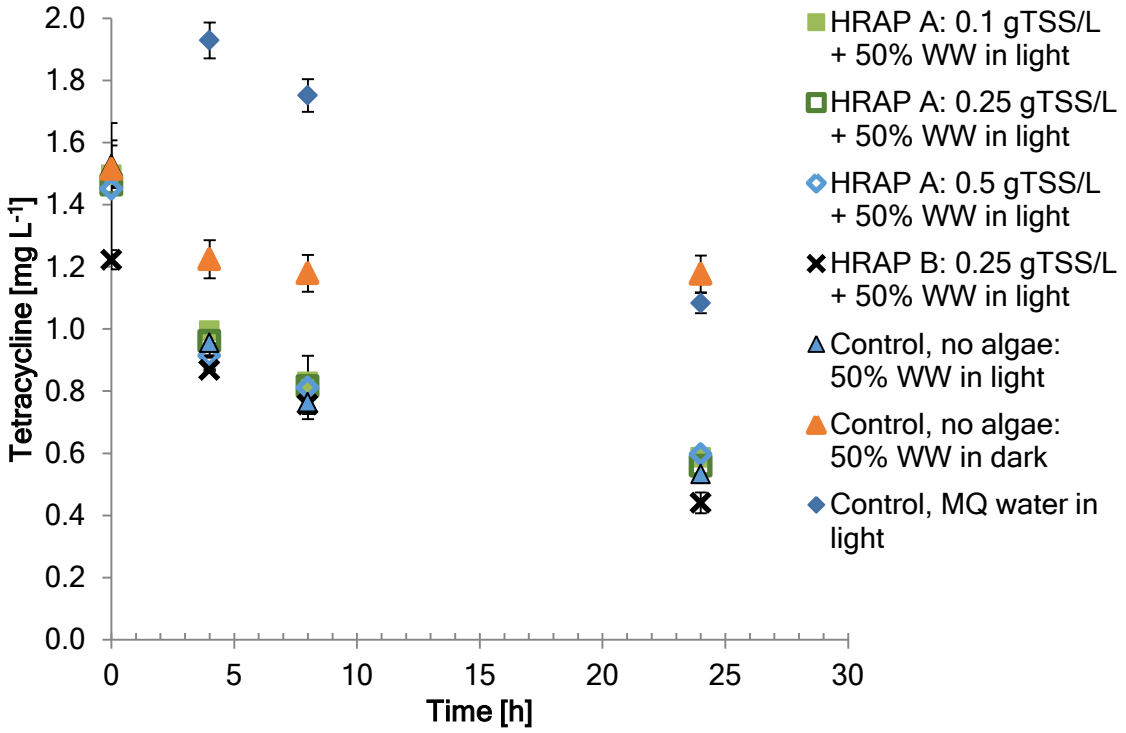

Figure 10: Tetracycline solutions degraded in light with varied biomass concentrations. Batch run 2nd Sep, using biomass from the lab HRAPs A & B, under 4 day HRT operation and 12 h light (Stage II). Error bars show the range of the two replicates for all samples except for the MQ water control and the 50% WW control in the dark, which had one replicate and error bars show typical analysis error (see Table 15).

In Figure 10, TET removal rates in the light (k_1 = 0.95 ± 0.06 d^{-1} (R^2 = 0.84)) were less than half the TET removal rate measured in the other batch trial reported in Table 18, which was also conducted during Stage II (k_1 = 2.6 ± 0.2 d^{-1} (R^2 = 0.97)).[a] Also, the TET removal rate in Figure 10 was only 4.5 times the removal rate in the dark control

[a] The kinetic rates were statistically different, with $p < 0.01$

($k_1 = 0.21 \pm 0.15\ d^{-1}$ ($R^2 = 0.51$)). This reduction in photodegradation was most likely due to the presence of WW organics – due to either competitive light absorption by the organics, or competitive quenching of generated reactive oxygen species.[a] This effect that the presence of WW organics had on photodegradation may have prevented the change in the algal biomass concentrations from causing any observable effect upon TET removal rates in this batch experiment. Similarly, the difference between the experiment by de Godos et al. (2012) and the one reported in Figure 10 is most likely due to differences in the WW used.[b] As expected, TET concentration slowly decreased in MQ water controls exposed to light, and the TET concentration in the no-algae dark control decreased quickly and stabilised, indicating that sorption to the WW particulates occurred, but no biodegradation. [c]

Overall, the batch tests in this section have shown that under these conditions: 1) hydrolysis and biodegradation were negligible; 2) there was a low contribution to TET removal by sorption, especially in the dark; 3) photodegradation strongly dominated removal; 4) Indirect photodegradation was much greater than direct photolysis. These results also suggest that high concentrations of organics may inhibit indirect photodegradation by competitively absorbing light or quenching reactive oxygen species.[d]

Although biodegradation was shown to be negligible in these batch tests, this does not yet prove that there is no biodegradation in the continuous HRAP. Biodegradation may be discouraged in the batch test due the shock at changing conditions/increased TET

[a] reactive oxygen species are involved in the indirect photodegradation mechanism
[b] de Godos et al. used synthetic WW, and the experiment in Figure 10 used real WW, which would have different dissolved organic compositions. Differences in light supply may also have affected the results - de Godos et al. used a lower light intensity and closed glass flasks.
[c] The fact that the final TET concentration was similar in the dark control and the MQ water control is a coincidence, since different removal mechanisms were involved
[d] This last conclusion is tentative, since no further experiments were conducted to confirm the results (e.g. experiment using radical scavengers or analysis to measure the reactive oxygen species directly).

concentrations or due to the lack of established biofilms in the beakers. For example, Rühmland et al. (2015) reported evidence of pharmaceutical biodegradation in facultative ponds, but no observed biodegradation during in-situ experiments in glass tubes. In other HRAP systems, different species composition or increased SRT from recycling biomass may also enable biodegradation to become significant.

In addition, the low direct photolysis observed in these batch experiments may not be representative of outdoor conditions. Direct photolysis is highly dependent on the wavelengths of light absorbed by the target molecule, as the TET absorbs UVB light (peak ~360 nm). The UVB component of the light used in these batch experiments (0.05%, Section 3.3.1) is lower than typical sunlight (2.4% UVB/ambient light). Literature reports disagree on whether indirect or direct photodegradation is dominant for TET, and comparisons are difficult due to the different experimental designs. In experiments with simulated sunlight (150 W xenon arc lamp), Chen et al. (2008) reported that there was a negligible increase in TET photodegradation rates following addition of nitrate ($N\text{-}NO_3^-$ = 14 mg L^{-1}) or humic acid (5 mg L^{-1}) to water, and hypothesized that direct photolysis of TET was the predominant degradation process in natural waters. However, organic concentrations in HRAP far exceed 5 mg L^{-1}. In contrast, López-Peñalver et al. (2010) reported that TET photodegradation rates significantly increased from $k_1 = 18 \pm 2\times10^{-3}$ min^{-1} in pure water to $k_1 = 35.5 \pm 0.9\times10^{-3}$ min^{-1} in WW and $k_1 = 59 \pm 2\times10^{-3}$ min^{-1} in surface water, which suggests that indirect photodegradation of TET occurred in the WW and surface water tests. However, López-Peñalver et al. (2010) used a low-pressure mercury lamp (254 nm), which was not representative of natural sunlight. Therefore further batch tests under natural sunlight were required, to test the findings of the indoor batch experiments under more relevent conditions.

4.3 Batch Experiments – 2.5 L

In the previous section it was concluded that indirect photodegradation was the dominant TET removal mechanism during indoor 100 mL batch experiments (Section 4.2). However, the intensity and spectrum of the light source may have a major impact on photodegradation mechanisms (Niu et al., 2013), and therefore the findings about TET removal mechanisms with artificial light needed to be confirmed during tests with exposure to natural sunlight. The results presented in this section were obtained during batch experiments conducted under natural sunlight in Valladolid, Spain, using HRAP biomass sourced from the 180 L pilot-scale HRAP, described in Section 3.3.3. These experiments were conducted in 2.5 L 'batch reactors' (145 mm liquid depth; see Section 3.4.2) at a similar liquid depth as the HRAP (150 mm deep) to maintain similar light attenuation in the batch tests compared with the HRAP.

In addition to testing the findings about TET photodegradation under natural sunlight exposure, the relative contribution of sorption and biodegradation needed to be quantified for the 180 L pilot HRAP. The WW characteristics and the algal species identified in the 180 L HRAP were different to the 7 L lab-scale HRAP (See Sections 4.1.1 and 4.4.2), and the different biology and growing conditions could enable biodegradation or change the sorption interactions between TET and the HRAP biomass.

Therefore, the results from full-day outdoor batch tests used to assess the relative contribution of TET removal mechanisms (sorption, biodegradation, and photodegradation) are discussed, followed by results from other batch tests specifically assessing the sorption removal mechanism, and the effect of pH upon TET removal.

4.3.1 Full day outdoor batch experiments

In total, five full-day outdoor batch tests were conducted to assess TET removal mechanisms under natural sunlight exposure. In addition to the variations in weather conditions for each batch test (e.g. air temperature, sunlight intensity) there were also changes in the biomass/effluent characteristics, since the biomass used in the batch tests was sourced from the pilot HRAP during different operational stages.

Three of the full-day batch tests will be discussed in this section:[a] two batch tests performed a week apart during 7-day HRT operation of the pilot HRAP, before any TET was supplied in the WW influent (28th May and 1st June 2015); and one full-day batch test was performed after 15 days of HRAP operation at 4-day HRT, with TET supplied continuously in the WW influent (5th Aug 2015). The results from the full-day batch test performed 1st June 2015 will first be discussed from Figure 11 in order to see the overall trends in TET removal. Then, the differences in TET removal rates will be quantitatively discussed based on the kinetic removal rates calculated for each of the three batch experiments listed in Table 19.

Each full day TET removal batch test began after sunset and continued through the night and the next day (22-26 h), to assess the TET removal under dark and light conditions. In Figure 11, three conditions are shown: the presence of active and autoclaved biomass, and the MQ water control. For the MQ water control, no hydrolysis, sorption to the plastic batch reactors, or other TET removal was observed

[a] Of the other two full-day batch tests with TET performed, one outdoor batch test was performed during 7-day HRT operation of the pilot HRAP, with TET supplied continuously in the WW influent. This test is not discussed further in this section, as a rapid initial decrease in detected TET occurred (probably due to wastewater characteristics), preventing direct comparison with the other batch tests. This test is discussed in Appendix Three Section S3.1, along with the possible reasons for the initial TET decrease.
In the final full-day batch test, TET removal was assessed as part of a mixture of three antibiotics, and the results are therefore discussed in Section 4.7 with the rest of the results from the antibiotic mixture experiments. This final full-day batch test with TET was performed during 4 day HRT operation of the pilot HRAP.

during the night. TET photolysis was observed during sunlight exposure, and TET photolysis appeared to be greater under sunlight than the previous results under artificial light (Section 4.2). This increased TET photolysis was most likely due to the proportionally higher UVB/PAR of the sunlight compared with artificial light.

In the case of autoclaved biomass (Figure 11), biodegradation was prevented by the autoclaving and no abiotic TET removal occurred in the MQ water control. TET removal in the presence of autoclaved biomass therefore appears to be via TET sorption to the biomass during the night, then photodegradation and potentially desorption[a] during the day.

Figure 11 provides evidence that TET removal during the night is higher for the active biomass than the autoclaved biomass, which may at first suggest TET biodegradation. However, most of the difference in TET aqueous concentrations appears to be due to the initial decrease within 1 hour, which suggests that rapid sorption occurred, rather than biodegradation. The differences in sorption between the active and autoclaved biomass may be caused by the disruption of the algal-bacterial biomass by autoclaving, or by differences in pH (batch tests with autoclaved biomass had a higher pH than with active biomass during the night; Figure 11). A separate batch experiment (Section 4.3.2) confirmed that the difference in TET removal between the active and autoclaved biomass conditions at night could be explained by sorption differences.

[a] Unless TET desorption is irreversible, then the TET that sorbed to the biomass during the night should desorb, returning to the aqueous phase as the photodegradation reduces the aqueous TET concentration.

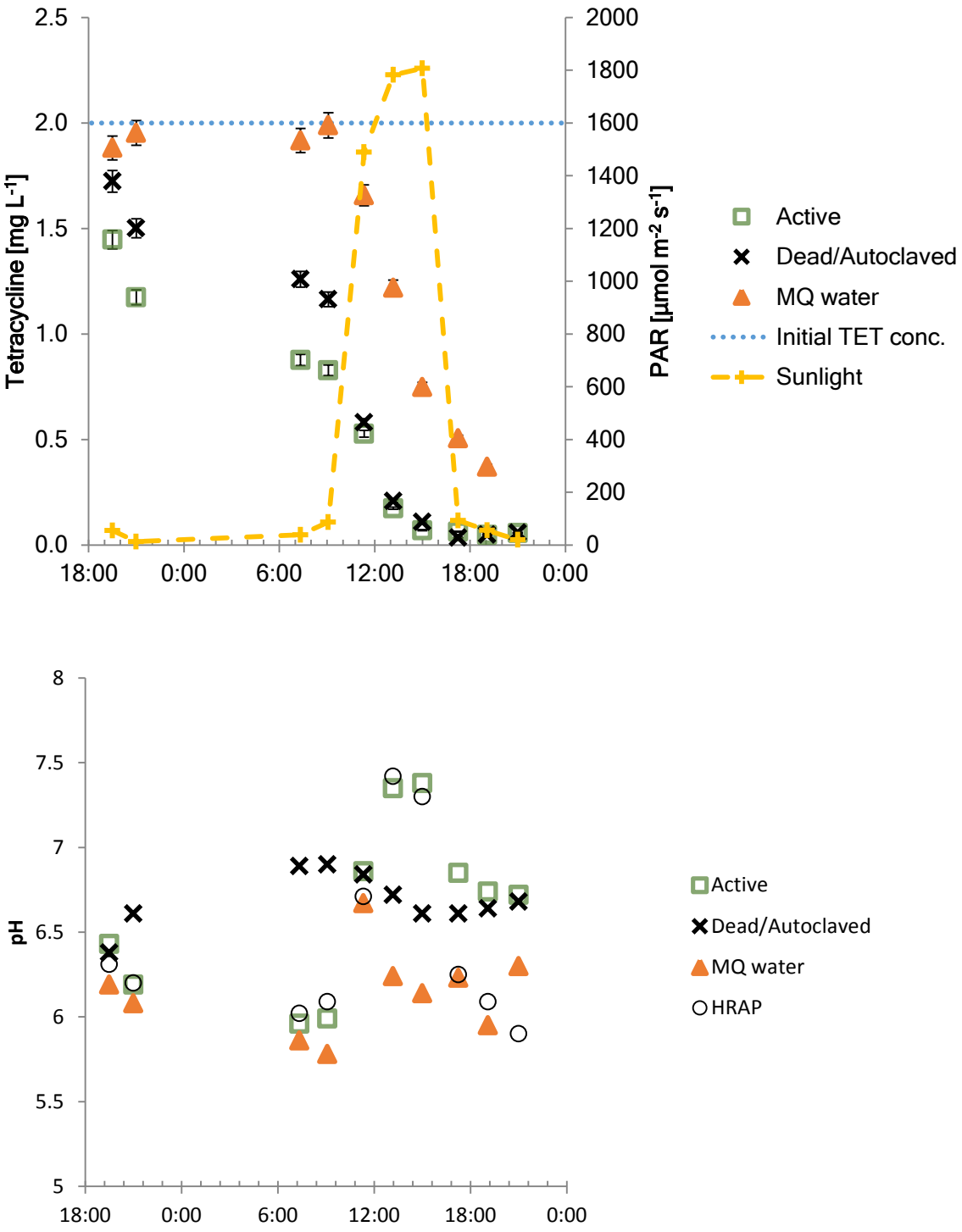

Figure 11: Batch tetracycline removal over a 24 hour period outdoors, beginning at night, 1st June August 2015. Error bars shown are typical analysis error (see Table 15).The time period covers an initial dark period overnight and then sunlight the next day. Sunlight intensity is shown as yellow dashed line. The pH values in the batch reactors and the 180 L HRAP (for comparison) are also shown.

As intended, diurnal fluctuations in the pH during the batch tests were similar compared with the pilot HRAP, although after 3 pm the pH decreased faster in the pilot HRAP than in the batch reactors (Figure 11). Temperature during sunlight exposure was slightly higher in the batch reactors (peak 40°C; Appendix Three) than in the pilot HRAP (peak 31°C; Appendix Three).

Although ~60% removal of the aqueous TET concentration was observed during the night by sorption to the active biomass (Figure 11), TET photodegradation still appears to be the dominant removal mechanism, as:

- TET removal by sorption stops when the sorbed concentration is at equilibrium with the aqueous TET concentration, but photodegradation should decrease TET concentrations until all TET is removed.
- As photodegradation decreases aqueous TET concentrations, sorbed TET should then desorb, until aqueous and sorbed TET concentrations reach equilibrium.

Having now seen the overall trends of TET removal in the batch experiments through the example in Figure 11, the pseudo-first order kinetic constants (k_1) for the TET removal across three different experiments are listed in Table 19. For the last batch experiment listed in Table 19 (5th August), additional conditions were used to test the effects of biomass concentration and shading effects.

Three main conclusions are clear from the data in Table 19:

- TET removal by photodegradation was the dominant removal mechanism. A comparison between the k_1 values listed in Table 19 during the night and the day shows that TET removal in the light is at least 10 times higher than the TET removal in the dark after the initial rapid decrease due to sorption. In the

presence of biomass during the night (9 pm-9 am), k_1 values were between 0.25-1.0 d^{-1}, and the k_1 values during the day (11 am-3 pm) were between 11-29 d^{-1}. Therefore, especially since the sorbed TET should desorb as aqueous TET concentrations decrease, it appears that photodegradation was the dominant TET removal mechanism.

- Direct photolysis of TET in natural sunlight was greater than with artificial light. The k_1 for TET removal in the light (11 am- 3 pm) was k_1 = 4.7-5.2 d^{-1} (Table 19) compared to the kinetic constants observed in the 100 mL batch tests (k_1 = 0.1 to 0.3 d^{-1}; Section 4.2). After adjusting for surface area, volume, and PAR light intensity, the TET removal by direct photolysis in the batch tests is still approximately 6 times higher under natural sunlight than under artificial light, likely due to the greater UVB light in natural sunlight.
- TET removal by indirect photodegradation was greater than by direct photolysis. Since there are no dissolved organics other than the TET in the MQ water control, only direct TET photolysis can occur. Dissolved and suspended solids absorb and block light, thus we should expect a decrease in direct photolysis of TET in the presence of biomass or HRAP effluent. Yet the absorption of light contributes to indirect photodegradation via and production of reactive oxygen species.[a] Since the k_1 values in the presence of biomass under light conditions (k_1 = 11-29 d^{-1}) are 2-6 times higher than the k_1 values for MQ water in the light (k_1 = 4.7-5.2 d^{-1}),[b] this supports the previous findings (Section 4.2) that TET removal occurred by indirect photodegradation.

[a] Ideally, the involvement of reactive oxygen species should be confirmed by direct measurement or use of scavengers. However, indirect photodegradation (see Section 2.5) is evidenced by these results.
[b] For each batch experiment, the difference in kinetic rates calculated based on TET removal in the presence of biomass or TET removal in the presence of MQ water were statistically significant ($p<0.05$)

Table 19: Summary of pseudo-first order kinetics during outdoor batch experiments, with R^2 values given in parentheses.

First order removal rates at night, 9 pm-9 am [d^{-1}]	**Active HRAP 100%**	**Active HRAP 10% in Effluent[a]**	**Dead HRAP 100%**	**Effluent Filtrate**	**MQ Water**	**Initial TSS [g L^{-1}]**
May 28th 2015	0.64 ± 0.12 (0.88)		0.70 ± 0.03 (0.96)		0.41 ± 0.14 (0.52)	1.27
June 1st 2015	0.69 ± 0.01 (1.00)		0.47 ± 0.06 (0.98)		0 ± 0.05 (n.a.)	1.33
5th August 2015	0.97 ± 0.03 (1.00)	0.25 ± 0.06 (0.89)	0.39 ± 0.08 (0.93)	0.59 ± 0.01 (1.00)	0.24 ± 0.01 (1.00)	1.10
First order removal rates in daylight, 11 am-3 pm [d^{-1}]	**Active HRAP 100%**	**Active HRAP 10% in Effluent**	**Dead HRAP 100%**	**Effluent Filtrate**	**MQ Water**	**Average sunlight intensity [µmol m^{-2} s^{-1}]**
May 28th 2015	19.3 ± 0.9 (0.98)		13.5 ± 0.4 (0.99)		4.7 ± 0.1 (1.00)	1509
June 1st 2015	13.2 ± 0.4 (1.00)		10.9 ± 0.9 (0.98)		5.2 ± 0.4 (0.98)	1693
5th August 2015	23.1 ± 3.7 (0.95)	29.2 ± 6.8 (0.90)	11.9 ± 0.2 (1.00)	23.0 ± 3.3 (0.96)	4.9 ± 0.3 (0.99)	1508

[a] This sample was added for the experimental trial on the 5th Aug, where 0.25 L of HRAP culture,1.20 L of clarified effluent and 0.05 L TET stock solution was used to create a test with approximately 10% of the TSS of the 'Active HRAP 100%'.

In addition to the three main conclusions listed above from the data in Table 19, several other observations were made:

- For two of the batch experiments in Table 19 (28th May 2015 & 5th Aug 2015), TET removal was observed for the MQ water controls in the dark, in contrast to the results for the batch performed 1st June 2015. However, this TET removal appears to be due to either reversible hydrolysis or epimerisation of TET, as results from other experiments showed that full recovery of the initial 2 mg L^{-1} TET could be obtained for MQ water controls in the dark when HPLC samples were pre-treated with citric acid and EDTA. This is discussed further in

Appendix 3, Section S3.13, along with the relevant experiments. When the HPLC pre-treatment was applied to the samples for the batch test 5th Aug 2015, the only samples affected were from the 'MQ water' control – the detected TET concentrations for the other four conditions with HRAP biomass did not change. Therefore, it was concluded that while some TET removal was observed, *irreversible* abiotic TET removal was still negligible in the dark.

- A comparison of the k_1 values (Table 19) during the night for the 'Dead HRAP 100%' and the 'Active HRAP 100%' shows that for two of the three batch tests, TET removal during the night was higher for the active biomass, even after the initial decrease TET concentrations by 9 pm. This increase in TET removal for the active biomass is most likely due to sorption which had not yet reached equilibrium, but slow biodegradation cannot be excluded. If biodegradation did occur, it was still negligible compared with sorption or photodegradation.
- Indirect photodegradation appeared to be reduced in the presence of autoclaved biomass vs. the presence of active biomass (all three batches, comparing k_1 values for 'Dead HRAP 100%' and the 'Active HRAP 100%' 11 am-3 pm). Visual observations suggested that the autoclaving disrupted the biomass flocculation, and increased light attenuation – blocking/absorbing more light and thus reducing photodegradation.
- The presence of high TSS concentrations also appeared to reduce indirect photodegradation: for the batch test on the 5th August 2015, the pseudo-first order kinetic constants between 11 am-3 pm were lower for 'Active HRAP 100%' (initial TSS = 1.1 g L^{-1}, k_1 = 23.1 ± 3.7 d^{-1}) than for 'Active HRAP 10%' (initial TSS = 0.11 g L^{-1}, k_1 = 29.2 ± 6.8 d^{-1}), although the difference was not

statistically significant ($p = 0.63$).[a] In contrast, the pseudo-first order kinetic constant for 'Effluent filtrate' (initial TSS = 0 g L^{-1}, $k_1 = 23.0 \pm 3.3$ d^{-1}), was similar to the pseudo-first order kinetic constant for 'Active HRAP 100%', and this appears to contradict the finding that high TSS concentrations reduced indirect photodegradation. However, the lower TET photodegradation in 'Effluent filtrate' compared with 'Active HRAP 10%' may instead be due to lower pH and /or DO concentrations during sunlight, due to less algal photosynthetic activity. The pH for 'Active HRAP 100%' and 'Active HRAP 10%' increased to 9.1 and 9.5 respectively, but the pH for 'Effluent filtrate' only increased to 8.4 during the day. Similarly, the DO concentrations for 'Active HRAP 100%' and 'Active HRAP 10%' increased to ~15 mg L^{-1} during the day, but the DO concentration for 'Effluent filtrate' only increased to 10.6 mg L^{-1}.

Therefore in summary, the full-day outdoor batch experiments have shown that: 1) TET photodegradation was dominant under these conditions; 2) direct photolysis by MQ water was higher under sunlight than under artificial light; 3) TET indirect photolysis was dominant over other TET removal mechanisms.

In addition, based on the results we suggest that: 1) increased TSS concentrations may reduce TET photodegradation; 2) Increased light attenuation due to disrupted biomass flocculation may decrease TET photodegradation; and 3) increased pH or DO concentrations during sunlight exposure may contribute to increased TET removal. Further experimental work is needed to test these suggestions.

A comparison of the kinetic TET degradation rates is shown in Section 4.6, where scaling factors and light intensity are taken into account during comparison

[a] The low sample size (n=3) incurs a large uncertainty– these experiments were conducted to verify findings at a scale more relevant to full-scale conditions, rather than analyzing a large number of replicates.

4.3.2 Sorption batch experiments

Two batch tests were conducted to help confirm whether sorption was the main removal mechanism during the night period and to quantify sorption kinetics and sorption isotherm constants for predicting TET removal in other systems. Although the contribution of sorption to overall TET removal was minimal in the continuous HRAPs (see Section 4.1 and later in Section 4.4), the batch test results (Section 4.2 and 4.3.1) demonstrated that sorption plays a major role in the absence of photodegradation: this removal mechanism must therefore be considered to understand the fate of TET in algal WWT ponds.

During the first sorption experiment (June 22nd 2015, shown in Figure 12) the amount of TET removal after 16 hours of gentle mixing in the dark correlated well with biomass concentration, with an average 0.97 mg TET removed for every gram of active biomass and 0.60 mg TET removed per gram of autoclaved biomass. Between 80-115% of the aqueous TET removed from solution was recovered by extracting sorbed TET from the biomass (Figure 12), supporting the hypothesis that sorption is the dominant removal mechanism in the dark and biodegradation is negligible.[a] The variation in recovery is most likely due to analytical and experimental errors at the low analyte concentrations.

[a] Due to the analytical uncertainty and variation in recovery, we cannot exclude biodegradation as a slow TET removal mechanism. However, if biodegradation does occur, it is minor compared with sorption under these conditions.

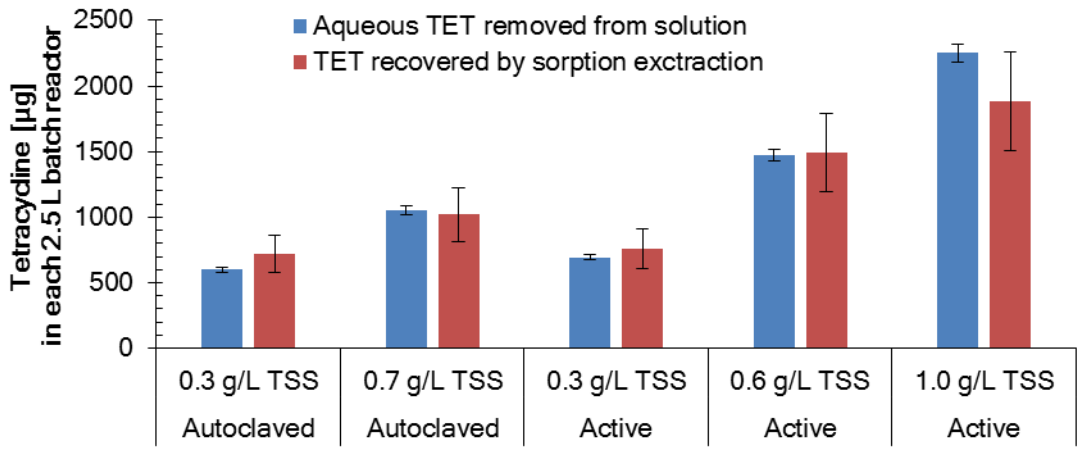

Figure 12: Tetracycline removal by sorption to active or autoclaved biomass at different biomass concentrations , after 16 hours incubation in the dark, 22nd June 2015. Error bars show the average analysis error (see Table 15).

At low TET concentrations, we would expect sorption equilibrium to be described by a partition coefficient $K_d = q/C$ where q is the sorbed biomass concentration [mg g_{TSS}^{-1}], and C is the aqueous TET concentration [mg L^{-1}] (Limousin et al., 2007).[a] To describe the TET sorption isotherm observed at 14 h, constants were fitted to the Freundlich and Langmuir isotherm models (Freundlich, 1906; Langmuir, 1918), as shown in Figure 15. The data was best described by the Freundlich isotherm ($R^2 = 0.999$),[b] which accounts for sorption behaviour occurring to sites with different sorption affinities (Mehta and Gaur, 2005). However, we have continued to use the partition coefficient approximation to estimate TET sorption in this thesis, as:

- We are only interested in TET sorption at low concentrations. As shown in Figure 15, a partition coefficient fitted to the three lowest-concentration data points was very similar to the Freundlich isotherm.

[a] The partition coefficient K_d is based on a simplification of the Langmuir sorption isotherm at low concentrations.
[b] The Langmuir isotherm had a poor fit to the data ($R^2 = 0.75$). The Langmuir isotherm describes mono-layer sorption to a surface of constant sorption affinity.

- Both the partition coefficient approximation and the Freundlich isotherm are ultimately empirical fits to the data (in contrast to the Langmuir isotherm, which has a theoretical basis), and thus must be confirmed every time experimental conditions change. The partition coefficient requires only one constant to be calculated, making it the simpler model, and more useful for comparison of data where there are only one or two data points (such as the TET sorption detected during continuous TET supply, Sections 4.1.2, 4.4.1, and 4.4.2).

The partition coefficient thus describing TET sorption for the three lowest-concentration data points was K_d = 1.4 ± 0.1 L g^{-1} (std. error of regression, n=3). This K_d value is similar to values observed during Stages I to III of the lab-scale HRAP monitoring (Section 4.1), and is within the range of K_d values reported for TET sorption to activated sludge (K_d = 1.1 L g^{-1} (Plósz et al., 2010), K_d = 8.4 L g^{-1} (Kim et al., 2005), K_d = 0.47 L g^{-1} (Prado et al., 2009)[a]).

The TET removal was best described by pseudo-first order kinetics (Figure 13; R^2 = 0.82-0.95), based on Equation 1 (Limousin et al., 2007):

$$\frac{dC}{dt} = k_{1s} * C - k_{1ds} * q * TSS \qquad (equation\ 1)$$

Where dC/dt is the rate of aqueous TET removal by sorption, k_{1s} and k_{1ds} are the pseudo-first-order kinetic constants for sorption and desorption[b] respectively (d^{-1}), C is the aqueous TET concentration (mg L^{-1}), q is the sorbed TET concentration (mg g^{-1}), and 'TSS' is the TSS concentration (g L^{-1}) of the HRAP.

[a] Prado et al. (2009) reported the Langmuir constant (L) and saturation concentration (Q_{max}), from which the sorption partition coefficient K_d was calculated by using the approximation $K_d = L \times Q_{max}$ at low TET concentrations.

[b] The kinetics of TET desorption from biomass was not experimentally determined, due to analytical difficulties at the antibiotic concentrations involved. Instead, the desorption kinetic rate (k_{1ds}) was calculated using equation 1 based on the experimentally measured values of k_{1s}, C and q at equilibrium (when $k_{1s}C = k_{1ds}qTSS$) .

The k_{1s} values were calculated based on the aqueous TET removal within the first hour, assuming that the rate of sorption was much greater than the rate of desorption during the first hour, and thus $k_{1ds} \approx 0$. At time $t = 0$, the theoretical initial aqueous TET concentration was used instead of the experimentally measured value. The kinetic constants thus calculated for the low-concentration TET data were $k_{1s} = 15 \pm 1$ d^{-1} (st.dev; n=4 initial TET concentrations 0.2-2 mg L^{-1}).[a] Precise sorption (and desorption) kinetics are best obtained using more advanced experiments (e.g. use of a stirred flow cell; Limousin et al., 2007). However, because the overall contribution of sorption to TET removal was small relative to photodegradation during the previous tests (Sections 4.1 - 4.3.1), this sorption kinetic constant should be suitable as a preliminary estimate for modelling the 180 L HRAP.

[a] The first order kinetics rates estimated at TET initial concentrations of 0.2, 0.5, 1, 2, 5, 10 mg L^{-1} were 13.4, 16.5, 14.0, 14.4, 10.1, and 6.5 d^{-1}, respectively. The rates appeared to be slower at higher initial concentrations, so these values were excluded, as lower concentrations were more relevant for the purpose. No obvious correlation was observed between the sorption kinetic rate and the initial concentration below 2 mg L^{-1}.

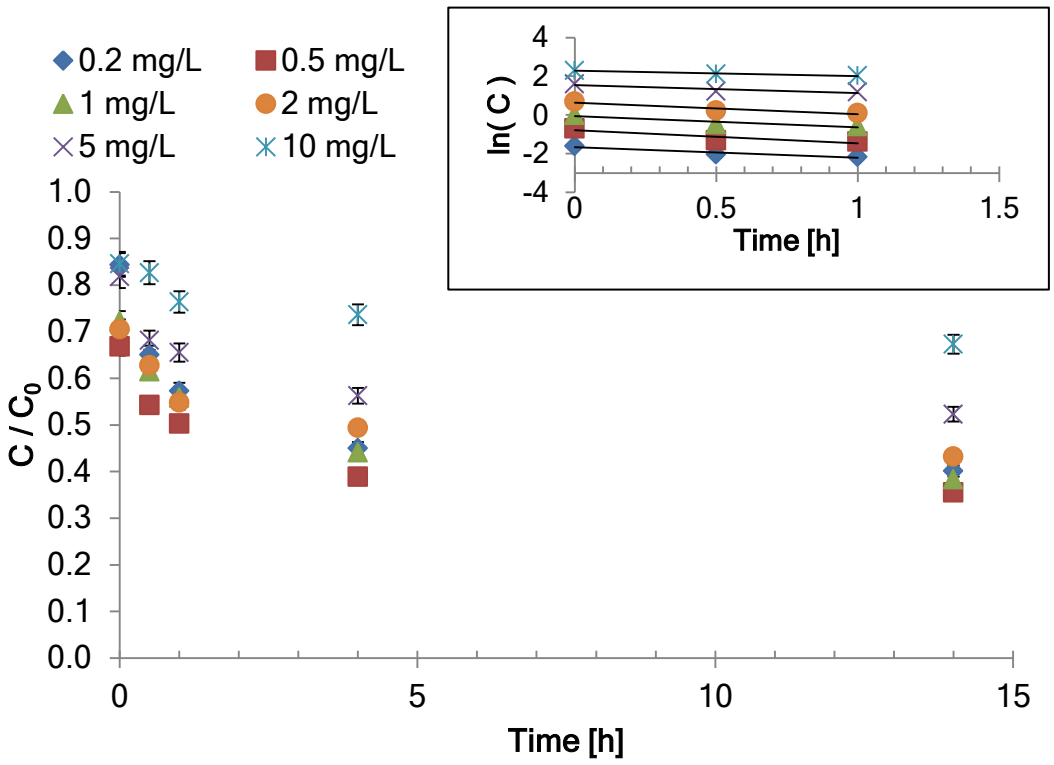

Figure 13: Batch removal of tetracycline at different initial concentrations to investigate sorption kinetics. Initial TET concentrations (C_0) are listed in the legend. TSS for all conditions was 0.95 ± 0.2 g L^{-1} (mean ± st.dev). Error bars show typical analysis error (see Table 15). Inset: regression of the pseudo-first-order kinetic constants used to describe TET removal by sorption during the first hour. [a]

[a] The linear trends support first-order kinetics, and the almost parallel trendlines in this plot of ln([TET]) vs. time show that the kinetic rate of sorption is similar in all the tests.

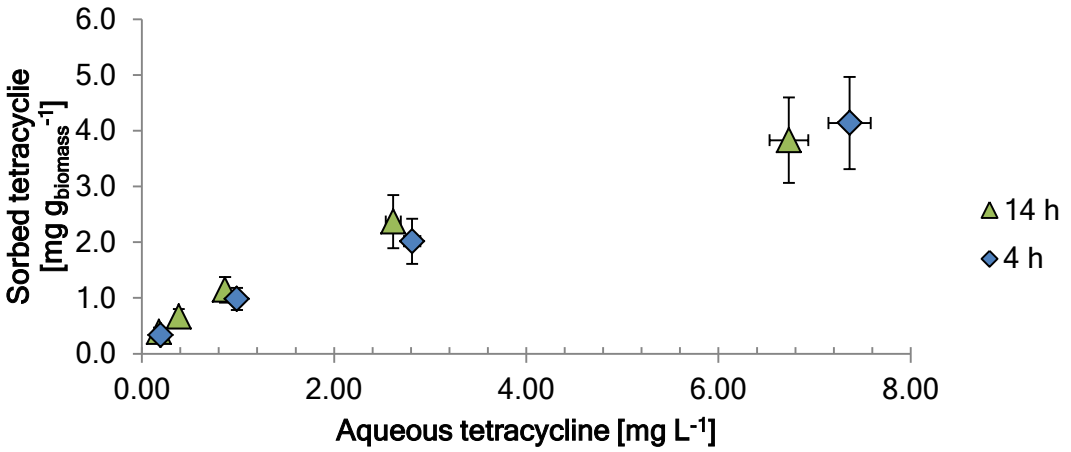

Figure 14: Sorption isotherm plot based on TET extracted from the biomass at 4 h or 14 h during the sorption batch experiment conducted Aug 31st 2015. Initial aqueous TET concentrations were 0.2-10 mg L^{-1}. TSS for all conditions was 0.95 ± 0.02 g L^{-1} (mean ± st.dev). Error bars show typical analysis error (see Table 15).

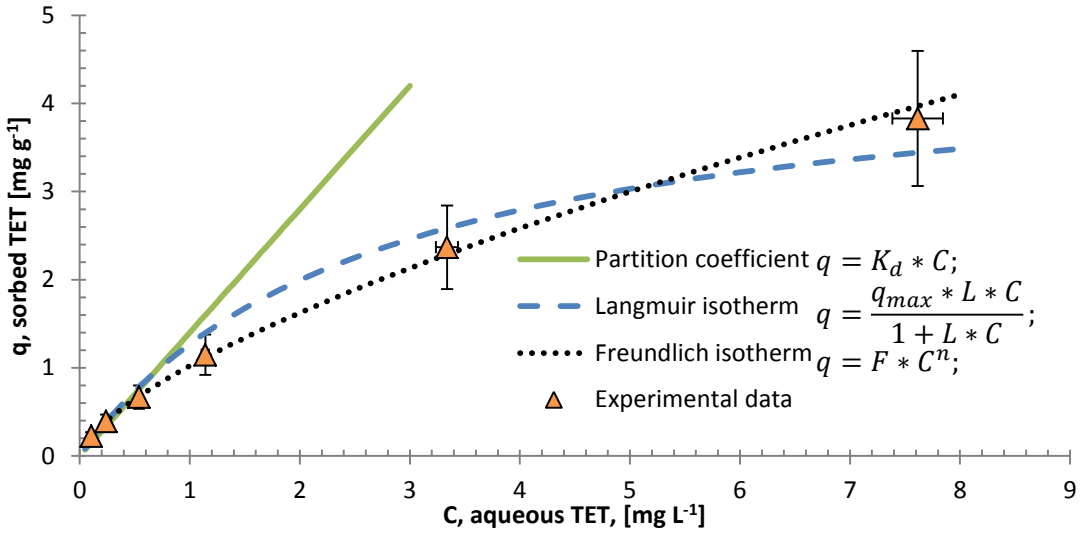

Figure 15: Comparison of the fitted sorption isotherm models[a] to the experimental aqueous and sorbed TET concentrations at 14 h during the sorption batch experiment (Aug 31st 2015). The 'partition coefficient' was fitted to the data below C = 1 mg L^{-1} only. Error bars show typical analysis error (see Table 15).

[a] Constants fitted for each isotherm were: for the linear isotherm, K_d = 1.4 L g^{-1}, for the Langmuir isotherm, L = 0.38 L g^{-1}, q_{max} = 4.64 mg g^{-1}; for the Freundlich isotherm F = 1.02 L g^{-1}, n = 0.69 (dimensionless).

4.3.3 pH batch experiments

During the full day outdoor batch tests (Section 4.3.1), pH was uncontrolled to keep conditions as similar as possible to the pilot HRAP. A 'pH batch experiment' was therefore conducted to investigate the effect of changing pH upon TET removal, independent of photosynthetic activity, as the structure of TET is dependent upon pH (Qiang and Adams, 2004). TET has pKa values around 3.3, 7.8, and 9.6 (see Section 3.2.1).

The pH in this batch experiment (4th August 2015) was manually adjusted to 6, 8.5, and 10.5 before each sampling point,[a] leaving one control without any pH adjustment (recorded pH was 6.5-6.8). The results for the TET removal during dark incubation[b] are shown in Figure 16, with full pH, DO, and temperature records are included in Appendix Three.

The removal of aqueous TET at pH 6 was not evidently different from the aqueous TET removal observed in the control with unadjusted pH. In contrast, increasing the pH to 8.5 and 10.5 caused an immediate drop in TET concentration, greatest at pH 10.5.

Sorption extraction tests were performed after 4 hours dark incubation. The measured sorbed TET was 0.29, 0.46, and 0.74 $mg_{TET}\ g_{TSS}^{-1}$ for the tests at pH 6, pH 8.5, and pH 10.5 respectively, with 0.31 $mg_{TET}\ g_{TSS}^{-1}$ for the uncontrolled pH control (pH 6.5-6.8). The increase in sorbed TET concentrations at pH 8.5 and 10.5 may be due to the addition of NaOH causing a release of extra-cellular polymeric substances (EPS) from the cells – this effect has been reported for activated sludge (Wu et al., 2010).

[a] These pH values were chosen as they are within a range relevant to algal ponds, and the dominant ionic species of TET should be different in each of the three pH adjusted conditions. Adjustment of the pH was made by addition of 0.5 M solutions of NaOH or HCl (Section 3.4.2.3)

[b] A test with pH adjustment of HRAP culture incubated under sunlight was also performed and is discussed later in this section. Although the pH usually drops at least below pH 8 at night in algal ponds, the effect of pH control was first tested in darkness to determine the impact of pH in the absence of photodegradation.

However, a mass balance of TET demonstrated that the increase in TET sorption at pH 8.5 or 10.5 was insufficient to explain the greater removal of aqueous TET removal observed. Even for the control with no pH adjustment, only 22% the aqueous TET removed from solution was recovered by sorption extraction. Yet experimental methods for the control in this experiment were the same as the methods for the sorption tests discussed in the previous section (Section 4.3.2) where full recovery of TET was obtained.[a] Even if biodegradation was occurring, it should not cause the rapid initial decrease in TET concentration observed and there is no reason why biodegradation should not have occurred in the previous sorption tests (Section 4.3.2). Therefore, the discrepancy in the mass balance can only be explained by either irreversible sorption (not recovered during sorbed TET extraction), or due to an unknown chemical reaction – perhaps TET epimerization or hydrolysis catalysed by a constituent in the HRAP effluent. Because we could not find a robust explanation for the fate of TET in these pH tests, the suggestion that higher pH caused increased TET sorption remains uncertain.

[a] The only difference between the pH batch tests and the sorption batch tests that we could find was that the biomass used for the pH tests was collected early in the morning (before sunlight reached the HRAP), while the biomass for the sorption batch tests was collected in the afternoon, when the HRAP had been exposed to sunlight for at least a couple of hours. However, no explanation could be found for how this change in the time that the biomass was collected could affect the TET removal so dramatically.

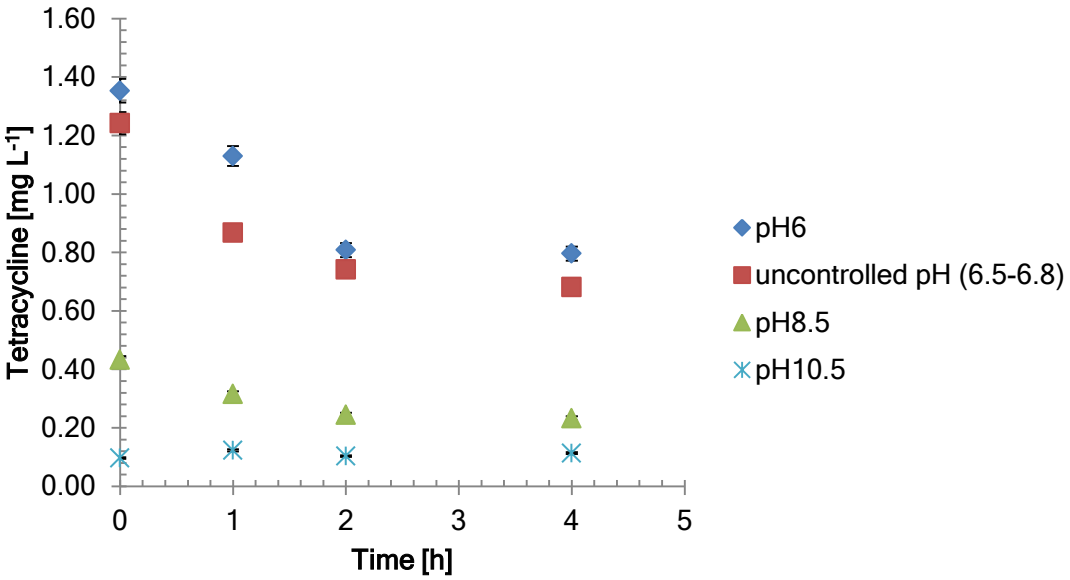

Figure 16: Tetracycline removal in a batch test conducted in the dark, 4th August 2015. pH adjustments were made by drop-wise addition of 0.1 M HCl or NaOH see Section 3.4.2.3). Error bars show typical analysis error (see Table 15). Average TSS was 0.99 ± 0.03 g L^{-1}.

A similar experiment was conducted outdoors to investigate the effect of independent pH adjustment upon TET removal during exposure to sunlight on 30th July 2015 (Appendix Three Section S3.10). Similar results were observed, with a rapid initial decrease in TET concentrations (within 2 min) under all conditions, and the initial decrease in TET concentration was greatest at pH 8.5 and 10.5. We therefore could not determine from these experimental results whether indirect photodegradation was affected by pH changes or not.

Overall, a high pH does appear to increase aqueous TET removal during treatment. The effect of high pH upon specific mechanisms of TET removal remains speculative due to uncertainties, but some data suggests that TET sorption to HRAP biomass increases at high pH.

4.4 Outdoor Pilot HRAPs

The fate of TET was studied in outdoor pilot scale HRAPs in NZ and Spain. This data is an important step forward in the relevance to full-scale conditions compared with the results presented thus far.

There were multiple differences between the two pilot scale HRAPs:

- Climate (Oceanic [NZ] vs. Mediterranean [Spain][a])
- Algal ecology (Dominant algal species were: *Pediastrum* sp., and *Scenedesmus* sp [NZ]; vs. *Acutodesmus obliquus* [Spain])[b]
- HRAP depth (0.26 m [NZ] vs. 0.15 m [Spain])
- HRAP construction material (concrete [NZ] vs. PVC [Spain])
- Average WW characteristics (e.g. COD was ~360 mg L^{-1} [NZ] vs. ~700 mg L^{-1} [Spain])
- Influent TET concentration (2 mg L^{-1} [NZ] vs. 100 µg L^{-1} [Spain])

The lower influent TET concentration used in Spain significantly increased relevance, as the TET concentrations in hospital WWs have been detected up to 158 µg L^{-1} (Pena et al., 2010). Domestic WW TET concentrations are typically around 1 µg L^{-1} (Michael et al., 2013), but detection at these TET concentrations requires both solid phase extraction (SPE) and liquid-chromatography-mass spectroscopy (LC-MS) analysis, both of which are expensive and time-consuming. SPE methods were used to lower the quantification limits to 2 µg L^{-1} for the TET analysis for the pilot HRAP in Spain (See Section 3.6), but we did not have access to LC-MS analysis.

[a] Both climates fall within the overall category of 'Temperate climate'. Valladolid, Spain is classified as Csb and Palmerston North, NZ is classified as Cfb by the Köppen–Geiger climate classification system (en.climate-data.org), where 'C' stands for temperate/mesothermal climate, 's' stands for 'dry summer', 'f' stands for 'without dry season', and 'b' stands for 'warm summer'.

[b] see Appendix One for other algal species identified.

4.4.1 900 L Pilot HRAP – NZ

This section discusses results from the operation of the 900 L HRAP at 9 day HRT with TET supplied continuously with the WW influent at 2 mg_{TET} L^{-1} (see Section 3.3.2). The purpose of this experiment was to assess the fate of TET under these conditions, and to test whether the high TET removal obtained during lab-scale operation (Section 4.1) could also be obtained at a depth relevant to full-scale HRAPs and under natural sunlight. This research was conducted from Feb-Apr 2015, late summer to early autumn in Palmerston North, NZ.

Due to site access restrictions, monitoring HRAP performance was limited to grab samples in the morning 2-3 times weekly. The average TSS concentration in the HRAP was 420 ± 90 mg L^{-1}, with consistent HRAP performance of 69 ± 11 % TOC removal, and 74 ± 10 % TN removal.[a] Full monitoring data is shown in Appendix One, Section S1.2.

The results from the TET monitoring are presented in Figure 17. For about 1 month (3 HRTs) after TET supply began, typical TET concentrations were below the quantification limit (0.02 mg L^{-1}). After 23rd Feb 2015, detected TET concentrations peaked at 0.3 mg L^{-1}, and subsequently stabilised at 0.12 ± 0.05 mg L^{-1}, which equates to a removal efficiency of 94 ± 3% (mean ± st.dev, n=28; 7th March-28th April 2015). The only known significant change in monitored HRAP conditions that occurred around 23rd Feb 2015 was a sharp peak in nitrite (up to 18 mg L^{-1} $N\text{-}NO_2^-$ on 9th Feb) followed by a decrease to <0.1 mg L^{-1} $N\text{-}NO_2^-$ by 27th Feb. The co-occurrence of the two changes suggests a link between nitrification and TET removal, but there is insufficient data to conclude whether the two changes have a causal relationship. Nitrite concentrations

[a] Since no clarifier was installed for this HRAP, removals are reported based on unfiltered WW influent samples and filtered (1 µm) samples taken from the HRAP.

increased again after TET supply in the HRAP was stopped, which lends support to the hypothesis that TET addition may have inhibited nitrification. Various studies have reported the ability of activated sludge nitrifiers to biodegrade some pharmaceuticals (Kruglova et al., 2014; Suarez et al., 2010; Vader et al., 2000). Fernandez-Fontaina et al. (2012) reported that 'nitrifying biodegradation' was due to co-metabolic action of the ammonium monooxygenase enzyme. However, no other correlations between nitrite concentration and TET removal were observed either in the lab HRAPs (Section 4.1), or in the 180 L pilot HRAP during continuous TET supply (Section 4.4.2).

A sorption extraction test performed 24th March 2015 detected a sorbed TET concentration of 0.19 mg_{TET} g_{TSS}^{-1} with a partition coefficient of K_d = 1.3 L g^{-1}. Based on productivity of 67 g_{TSS} d^{-1},[a] the TET sorbed onto biomass in the effluent was estimated to be ~ 6% of the influent TET load.

Because site access restrictions prevented regular monitoring of TET concentrations in the afternoon, differences between light and dark removal mechanisms were not distinguished for the continuous monitoring. The differences were therefore investigated using two pulse tests performed in March and will be discussed in Section 4.4.3.1.

Overall, consistently high TET removal was observed during operation of this 900L HRAP, with similar TET effluent concentrations as the lab-scale HRAPs (Section 4.1). TET sorption was likely significant but minor, suggesting that photodegradation was the main removal mechanism if biodegradation was negligible (based on results of the batch tests in Section 4.2, which used similar algal-bacterial biomass).

[a] Productivity was estimated based on the TSS measured that day, multiplied by the estimated effluent flow-rate based on net evaporation from the HRAP (see Section 3.3.2)

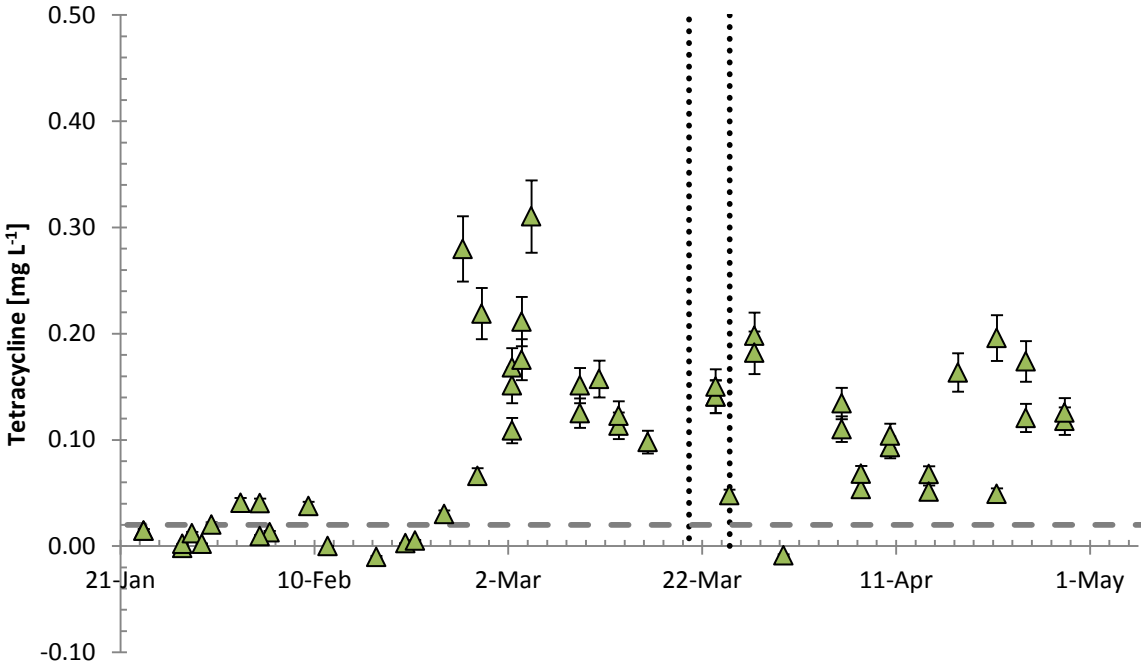

Figure 17: Removal of tetracycline in a 900 L HRAP with 9 day HRT supplied with 200 mg TET per day (~ 2 mg/$L_{influent}$). Error bars show analysis error (see Table 15). The dotted vertical lines indicate the dates for the TET pulse tests conducted in March (Section 4.4.3.1). The dashed horizontal line indicates the quantification limit of TET.

4.4.2 180 L Pilot HRAP (Spain)

This section presents the results from the monitoring of TET fate in the pilot-scale HRAP (180 L), conducted in Valladolid, Spain, from May-Aug 2015 (summer to early autumn). The main purpose of this experiment was to test whether high TET removal could still be achieved during continuous operation at lower influent TET concentrations (100 µg L^{-1}), along with other differences between the two pilot HRAPs. In contrast to the 900 L pilot scale HRAP in the previous section, a settler was used to clarify the effluent, and the effluent flowrates were monitored.

The HRAP was commissioned and operated without TET addition until the TSS concentration stabilised. Four main operational regimes could thus be distinguished: 1) 7-day HRT operation without TET supply; 2) 7-day HRT operation with TET added to the influent WW; 3) 4-day HRT operation with TET added to the influent WW; 4) 4-day HRT operation with high-concentration TET pulses to study the dynamic fate of TET.[a] HRAP performance was monitored at both 7-day HRT and 4-day HRT operation, as reported in Table 20. During all stages of operation, good average total COD removal (80 ± 4%) and total TOC removal (81 ± 4%) were observed[b]. When HRT was decreased from 7 day HRT to 4 day HRT, biomass productivity increased from 5.9 g_{TSS} d^{-1} to 18.2 g_{TSS} d^{-1} possibly due to higher inorganic carbon concentrations at 4 day HRT (Stage II). Total nitrogen and phosphate removals averaged 44 ± 12% and 45 ± 6 % respectively. HRAP performance was similar to reported performance for other HRAPs operated with WW (Park et al., 2013; Posadas et al., 2015a), confirming the relevance of this pilot-HRAP operation for the study of TET removal. Complete statistics on measured performance are shown in Appendix One, Section S1.3.

[a] Results from the TET pulse experiments are discussed in Section 4.4.3
[b] Total COD and TOC removal is reported for this HRAP, removal efficiencies are based on unfiltered effluent samples after the clarifier.

Table 20: 180 L HRAP performance and monitored environmental conditions during stable operation. Data is shown as mean ± 95% CI (n).

	Stage I (7 day HRT, no TET)			Stage II (7 day HRT, continuous TET)			Stage III (4 day HRT, continuous TET)			Stage IV (4 day HRT, continuous or pulsed TET)		
Characteristic	**Influent**	**Effluent**	**Removal Efficiency**	**Influent**	**Effluent**	**Removal Efficiency**	**Influent**	**Effluent**	**Removal Efficiency**	**Influent**	**Effluent**	**Removal Efficiency**
COD [mgO_2 L^{-1}][a]	787 ± 392 (6)	396 ± 112 (6)	75% ± 26% (5)	621 ± 63 (8)	373 ± 22 (10)	84% ± 2% (9)	669 ± 258 (3)	479 ± 241 (3)	78% ± 10% (3)	690 ± 104 (8)	407 ± 31 (8)	79% ± 5% (8)
N-NH_4^+ [mg L^{-1}]	56.3 ± 59.0 (6)	4.9 ± 5.1 (6)	95% ± 2% (5)	65.8 ± 47.1 (10)	13.7 ± 9.8 (10)	89% ± 2% (10)	71.7 ± 178.1 (3)	13.8 ± 34.2 (3)	85% ± 4% (3)	59.1 ± 49.4 (8)	11.7 ± 9.8 (8)	84% ± 3% (8)
N-NO_2^- [mg L^{-1}]	not detected	42.2 ± 44.3 (6)	n.a.	not detected	39.1 ± 27.9 (10)	n.a.	not detected	24.4 ± 60.7 (3)	n.a.	not detected	5.9 ± 5.5 (7)	n.a.
N-NO_3^- [mg L^{-1}]	not detected	3.2 ± 3.4 (6)	n.a.	not detected	30.1 ± 21.5 (10)	n.a.	not detected	16.2 ± 40.2 (3)	n.a.	not detected	33.9 ± 28.3 (8)	n.a.
P-PO_4^{-3} [mg L^{-1}]	9.7 ± 10.1 (6)	7.5 ± 9.3 (5)	51% ± 8% (4)	10.1 ± 7.2 (10)	8.9 ± 8.2 (7)	49% ± 6% (7)	10.1 ± 25.2 (3)	5.9 ± 53.0 (2)	59% ± 7% (2)	8.3 ± 7.7 (7)	7.3 ± 6.7 (7)	31% ± 11% (7)
Total Nitrogen [mg L^{-1}][a]	107 ± 26 (6)	58 ± 6 (6)	44% ± 60% (6)	102 ± 9 (9)	91 ± 16 (9)	47% ± 15% (9)	70 ± 20 (3)	54 ± 16 (3)	39% ± 24% (3)	93 ± 11 (8)	59 ± 11 (8)	46% ± 20% (8)
Total Organic Carbon [mg L^{-1}][a]	176 ± 44 (6)	32 ± 3 (6)	81% ± 19% (6)	165 ± 11 (9)	42 ± 5 (9)	88% ± 4% (9)	147 ± 113 (3)	44 ± 5 (3)	75% ± 20% (3)	171 ± 19 (8)	46 ± 11 (8)	77% ± 5% (8)
Inorganic Carbon [mg L^{-1}][a]	87 ± 19 (6)	12 ± 7 (6)	90% ± 5% (6)	87 ± 17 (9)	6 ± 1 (9)	95% ± 1% (9)	82 ± 7 (3)	17 ± 8 (3)	84% ± 8% (3)	88 ± 9 (8)	21 ± 8 (8)	81% ± 7% (8)
TSS [g L^{-1}][b]	0.12 ± 0.03 (6)	0.016 ± 0.004 (6)	89% ± 5% (6)	0.13 ± 0.02 (10)	0.022 ± 0.006 (10)	90% ± 3% (10)	0.11 ± 0.05 (3)	0.051 ± 0.022 (3)	63% ± 33% (3)	0.12 ± 0.04 (8)	0.057 ± 0.016 (8)	64% ± 16% (7)

[a] Concentrations and removal efficiency for these parameters (COD, TN, TOC, IC) are reported based using unfiltered effluent samples, collected after the clarifier.
[b] Removal efficiency for TSS was calculated based on influent and clarified effluent concentrations and their respective flows: it thus includes but is not limited to the settling efficiency of the clarifier.

Table 21 lists the normal environmental conditions and productivity data for the 180 L HRAP, as these conditions may affect the fate of TET. The average biomass concentrations in the HRAP remained similar during the four operational stages (TSS: 1.1-1.3 g L^{-1})[a]. The pH and DO concentrations during the day ('High pH', and 'High dissolved O_2', Table 21) were lower than expected based on typical values for HRAPs, where pH can increase to 11, and DO concentrations can increase up to 30 mg L^{-1} (Table 1, Section 2.2). This was most likely due to the high COD loads (Table 20), and the high TSS concentrations (Table 21)[b], which resulted in high biological respiration.

Table 21: Environmental parameters and productivity recorded for the HRAP during operation. The surface area of the HRAP was 1.2 m^2.

Stage		**I**	**II**	**III**	**IV**
TET supply		None	Continuous	Continuous	Continuous or Pulsed
Stable Period		26th May-16th Jun	17th Jun-21st Jul	29th Jul-6th Aug	6th Aug-3rd Sep
HRT	**d**	7	7	4	4
TSS	**g L^{-1}**	1.1 ± 0.3 (6)	1.1 ± 0.1 (10)	1.3 ± 0.6 (3)	1.2 ± 0.1 (8)
Low[a] pH	**[]**	5.5 ± 0.3 (17)	5.6 ± 0.1 (32)	5.9 ± 0.3 (8)	6.2 ± 0.1 (25)
High[a] pH	**[]**	6.5 ± 0.2 (17)	6.9 ± 0.2 (32)	7.5 ± 0.5 (8)	7.3 ± 0.2 (25)
Low[a] DO	**mg L^{-1}**	2.2 ± 1.5 (14)	0.6 ± 0.1 (33)	0.3 ± 0.1 (8)	0.2 ± 0.1 (28)
High[a] DO	**mg L^{-1}**	12.6 ± 0.8 (14)	9.8 ± 0.9 (33)	10.0 ± 2.8 (8)	8.0 ± 0.8 (28)
Low[a] temperature	**°C**	15.0 ± 2.2 (17)	16.5 ± 0.7 (33)	15.6 ± 1.2 (8)	15.3 ± 0.7 (28)
High[a] temperature	**°C**	25.9 ± 3.3 (17)	32.8 ± 1.0 (33)	32.4 ± 1.1 (8)	29.8 ± 1.6 (28)
Productivity	**g m^{-2} d^{-1}**	7.4 ± 5.3 (6)	4.5 ± 2.0 (10)	15.0 ± 1.7 (3)	15.9 ± 4.6 (8)
Average evaporation	**L m^{-2} d^{-1}**	2.9 ± 4.6 (21)	7.7 ± 2.2 (32)	10.4 ± 7.8 (10)	6.7 ± 2.3 (27)

[a] 'Low' and 'High' parameters refer to the 5th and 95th percentiles of 24-hour datalogged values: these percentiles are reported rather than max/min in order to remove potential outliers in the data.

[a] TSS concentration was stable despite higher productivity due to 1) higher effluent flows reducing the proportional influence of evaporation (10-12 kg d^{-1}) and decreasing the effect of partial-clarification of effluent occurring at the overflow outlet of the HRAP; 2) decreased biomass decay during long biomass retention times

[b] 0.3 g L^{-1} is more typical for a HRAP without biomass recycle, see Table 1

TET was added to the WW influent after allowing the HRAP TSS concentration to stabilize at ~1.1 g_{TSS} L^{-1}, and the resulting TET concentrations during continuous operation with TET are reported in Figure 18. TET was mixed with the influent WW in a feed tank refilled every one or two days.[a] During Stage II, TET concentration remained below the quantification limit (2 µg L^{-1}; Figure 18). During Stage III, the HRT was reduced to 4 days and TET effluent concentrations increased to an average of 4.0 ± 0.5 µg L^{-1} TET (95% CI, n=14, Figure 18) yielding a TET removal efficiency of 97 ± 1% (95% CI, n=14). There was a statistically significant difference between HRAP TET concentrations recorded in the morning (5.2 ± 0.9 µg L^{-1}; mean ± 95%CI, n=8) and evening (2.5 ± 0.5 µg L^{-1}; mean ± 95%CI, n=6) during 4 day HRT operation (Single-factor ANOVA, $p<0.01$). The lower concentration reported in the HRAP following sunlight exposure supports the hypothesis that photodegradation is the dominant TET removal mechanism under these conditions. Pulse tests (Stage IV, with results discussed in Section 4.4.3) were conducted to further differentiate and quantify TET fate in the day and night periods.

[a] Aqueous TET concentrations were sampled several times in the influent WW tank, after addition of the TET stock solution to create an intended 100 µg L^{-1} TET concentration in the influent. For freshly prepared WW (sampled at 0-2min), aqueous TET concentrations were 69 ± 9 µg L^{-1} (st. dev., n=15), and further decreased to 42 ± 6 µg L^{-1}(st. dev., n=3) by 10 min, but then stabilised with aqueous TET concentrations after 24 hours at 36 ± 2 µg L^{-1}(st. dev., n=5). The rapid initial decrease in aqueous TET concentrations is most likely due to sorption (the main removal mechanism for TET in activated sludge: Kim et al. 2005), which should not have no real impact on the main findings, as the sorbed + aqueous TET concentrations in the wastewater influent still equate to 100 µg L^{-1} TET.

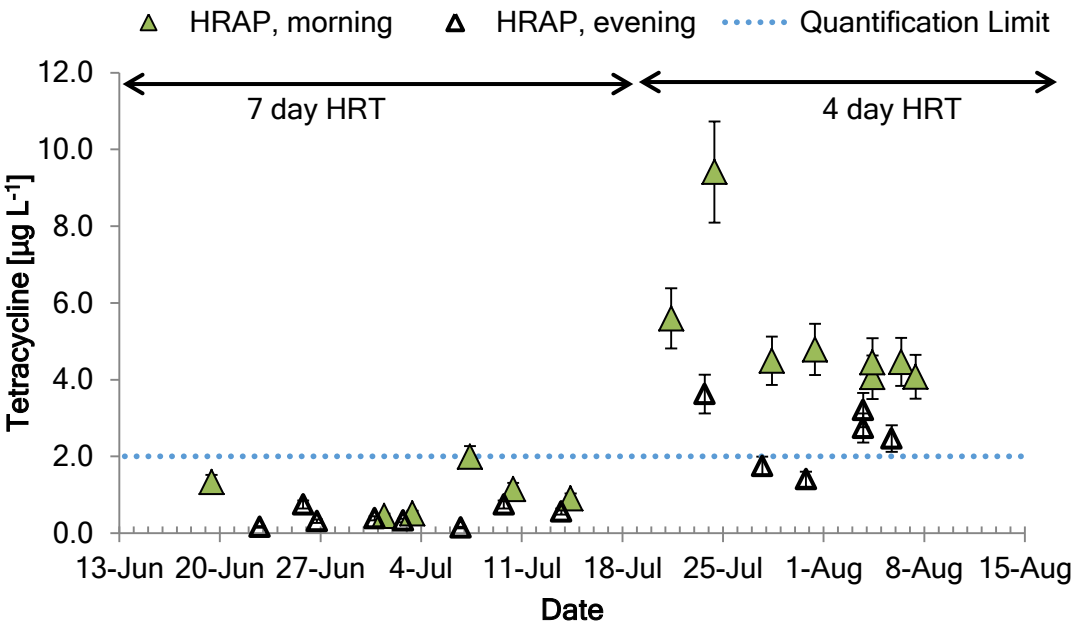

Figure 18: Tetracycline concentrations in the 180L HRAP at morning and evening sampling times with continuous 100 µg L^{-1} TET in WW influent. Error bars show typical analysis error (see Table 15), and the quantification limit is shown as a dotted horizontal line.

Sorption extraction tests were performed during Stage II, 7-day HRT operation, and TET sorption on HRAP biomass was estimated to be 4.0 to 4.1 $\mu g_{TET}\ g_{TSS}^{-1}$ (range, n=2)[a]. Based on the average productivity during Stage II (5.4 $g_{TSS}\ d^{-1}$, Table 21), the removal of TET by biomass sorption was estimated to be 1% of the influent TET load. During Stage III, 4-day HRT operation, TET sorption increased to 12.3 to 12.6 $\mu g_{TET}\ g_{TSS}^{-1}$ (range, n=2)[b]. Since the average productivity during Stage III also increased, (18.0 $g_{TSS}\ d^{-1}$)[c], the estimated TET removal by sorption increased to 5.3% of the influent TET load. This 5.3% TET removal by sorption was similar to the range of the

[a] TET sorption to the settled biomass in the clarifier was also measured (1.8 $\mu g_{TET}/g_{TSS}$). The lower TET sorption detected for settled biomass in the clarifier vs. the HRAP biomass may indicate that there was additional TET degradation in the settler, but the difference could also be due to analytical uncertainty.
[b] Detected TET sorption to the settled biomass in the clarifier was 7.0 $\mu g_{TET}/g_{TSS}$.
[c] Productivity of 15.0 g $m^{-2}\ d^{-1}$ (Table 21) × surface area 1.2 m^2.

TET removal by sorption observed during operation of the lab HRAPs (3.2-7.0% removal by sorption for Stages I to III, Section 4.1).[a]

Based on the rapid sorption kinetics observed in the batch tests, the TET sorption should be near equilibrium, allowing the calculation of an equilibrium partition coefficient (K_d). For the two sorption extraction tests conducted during Stage III, 4 day HRT operation, [b] the aqueous TET concentrations were 2.7 mg L^{-1} and 4.5 mg L^{-1}, with sorbed TET concentrations of 12.3 and 12.6 μg_{TET} g_{TSS}^{-1} respectively. Based on these concentrations, the K_d values were 4.6 L g^{-1} and 2.8 L g^{-1} respectively. These values are higher than the K_d observed in the batch test (K_d = 1.4 ± 0.1 L g^{-1}; Section 4.3.2). The difference between the values of K_d measured in the batch test and in the pilot HRAP is understandable given: 1) the known sensitivity of the partition coefficient to experimental conditions (Limousin et al., 2007); 2) the ~100-fold difference in TET aqueous and sorbed concentrations; and 3) analytical uncertainties in the sorbed and aqueous TET concentrations.

[a] The similar range of TET removal by sorption is due to a combination of lower biomass concentrations (therefore decreased sorption) and reduced overall TET removal efficiency (higher aqueous concentrations, therefore increased sorption) in the lab-scale HRAPs.
[b] A partition coefficient could not be calculated under 7 day HRT operation (Stage II), since aqueous TET concentrations were below the detection limit.

4.4.3 Pulse tests in Pilot HRAPs

This section discusses results from two pulse tests performed during the 900 L HRAP operation in NZ (~2 mg L^{-1} peak TET concentration), and a series of six pulse tests performed during the 180 L pilot HRAP operation in Spain (~100 µg L^{-1} peak TET concentration). These pulse tests were conducted in order to: 1) better resolve the differences between light and dark TET removal rates in pilot-scale HRAPs; 2) test how the system responded to shock loads of TET; 3) provide experimental data to test the validity of predictive modelling simulations (discussed in Section 4.4.3.3).

Pseudo-first-order kinetic rates were used to describe TET removal, since the batch test results (Section 4.2 & Section 4.3) indicated that first-order rates typically provided the best fit to explain TET removal. The decrease in TET concentration due to the effluent flow was accounted for in the calculation of pseudo-first order kinetic constants (k_1, d^{-1}),[a] the integrated rate equation for TET removal is:

$$C(t) = C_0 * exp\left(-\left(\frac{Q_{out}}{V} + k_1\right)t\right) \qquad (equation\ 2)$$

Where C is the aqueous TET concentration [µg L^{-1}], C_0 is the initial TET concentration [µg L^{-1}] after a pulse at time t=0, Q_{out} is the effluent flow-rate [L d^{-1}], and V is the volume of the HRAP [L].

[a] Assuming that the HRAP is well-mixed

4.4.3.1 900 L Pilot HRAP pulses - NZ

The results from the two pulse tests conducted in the 900 L HRAP are shown in Figure 19. These results showed that the HRAP could mitigate a large TET pulse quickly. The HRAP was operated at 9 day HRT, as described in Section 3.3.2, and the pulse tests were conducted during the continuous TET removal monitoring discussed in Section 4.4.1[a].

Although the theoretical initial increase in TET concentration was 2.2 mg_{TET} L_{HRAP}^{-1}, the TET concentrations in both tests peaked at ~1.2 mg L^{-1} after 15 min in the HRAP. It was uncertain whether the low initial increase in TET concentration after the pulse was due to TET sorption to the biomass and/or concrete walls, incomplete dissolution, or some other cause. Sampling was conducted from 7 am to 3 pm the following day.

Based on the measured TET concentrations, the rate of TET removal increased during sunlight exposure, thus supporting the hypothesis of photodegradation as a dominant TET removal mechanism, as seen in Figure 19.[b] During the pulse test conducted on the 19th March 2015, the pseudo-first-order TET removal rates[c] were 0.53 d^{-1} (n=3) during the night and 3.4 ± 1.0 d^{-1} (n=9) during the day. During the pulse test conducted on the 25th March 2015, the pseudo-first-order TET removal rates were k_1 = 0.73 d^{-1} (n=3) during the night and 3.6 ± 0.5 d^{-1} (n=11) during the day. The rates were therefore consistent between the two experiments.[d] Sunlight (PAR) reached a peak intensity of 2,070 µmol $m^{-2}s^{-1}$ on 20th March and 1,880 µmol $m^{-2}s^{-1}$ on 25th March, and TSS

[a] The influent TET supply was stopped during the pulse experiments, see Section 3.3.2
[b] Results from a similar experiment with ciprofloxacin in the same HRAP also showed that photodegradation was also a dominant removal mechanism for ciprofloxacin, see Section 4.7.1.
[c] To calculate the k_1 values, a constant effluent flow-rate of 90 L d^{-1} was estimated based on the open-water evaporation data for Palmerston North on the 21st March and the 26th March 2015 (clifo.niwa.co.nz). No rainfall was recorded on the days of these two pulse tests.
[d] There was no statistical difference (p = 0.66) between the light TET removal rates achieved during each experiment. Statistical testing on the difference in TET removal rates at night were not possible due to insufficient data points collected during the night period.

concentrations were 0.54 g L^{-1} on 20th March and 0.63 g L^{-1} on 25th March. Other monitored weather and HRAP conditions are shown in Appendix One Section S1.4.1.

As previously discussed in Section 4.4.1, the continuous TET supply appeared to have an effect on nitrification in the HRAP. However, the shock-loads of TET in these pulse experiments did not appear to have any additional effect upon HRAP performance. Furthermore, the shock-loads of TET did not appear to decrease the removal efficiency of TET when continuous supply of TET was resumed after the pulse experiments (see Section 4.4.1, Figure 17).

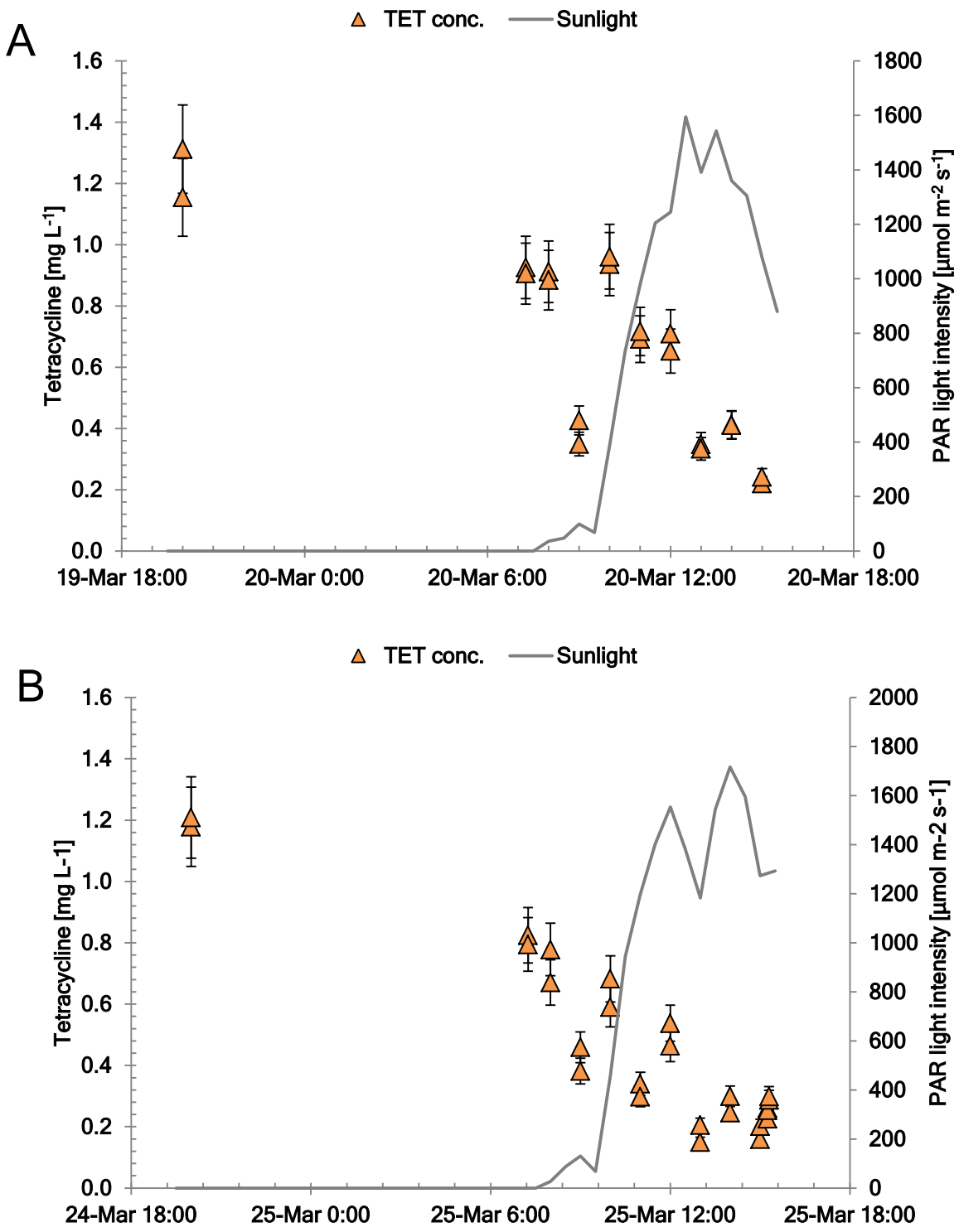

Figure 19: TET Pulse tests in 900 L HRAP, March 2015. Pulses of 2 mg_{TET}/L_{HRAP} were added at 20:00. Sunlight intensity data (continuous line) is shown as half-hour averages. A: 18th-19th March, B: 24th-25th March. Error bars show analysis error (see Table 15).

4.4.3.2 180 L Pilot HRAP pulse experiments – Spain

Six TET pulse tests were performed in the 180 L HRAP in Spain: the aqueous TET concentrations recorded are shown in Figure 20 and Figure 21, along with the light intensity recorded during the experiments. In four of these pulse tests, TET was added twice: once in the morning during direct sunlight exposure, and once after direct sunlight exposure stopped in the evening.[a] The other two pulse tests were performed with only one TET pulse addition (18th Aug 2015: in the morning; 31st Aug 2015: in the evening). For each TET pulse, 18 mg TET was added (180 mL of 100 mg L^{-1} stock solution), to raise the concentration of TET in the HRAP by 100 µg L^{-1}. Short-circuiting was prevented by closing the HRAP outlet for 5 min following the addition of the TET pulse[b]. The pulses were all conducted during 'Stage IV' of the HRAP operation at 4 day HRT, after the continuous monitoring described in Section 4.4.2. Further monitoring data (pH, DO, temperature) is shown in Appendix One Section S1.4.2.

The theoretical initial TET concentration was never detected after TET addition as the observed increase in TET concentration varied between 57-87 µg L^{-1}, instead of a 100 µg L^{-1} increase, and for the 18th Aug only a 26 µg L^{-1} increase in TET concentration was observed. These initial TET concentrations were similar to the results observed during batch tests, most likely due to rapid TET sorption during the initial 5 min mixing.

[a] The HRAP was shaded by surrounding buildings/trees in the evening.
[b] TET-free WW influent flow continued throughout the pulse experiments (see Section 3.3.3).

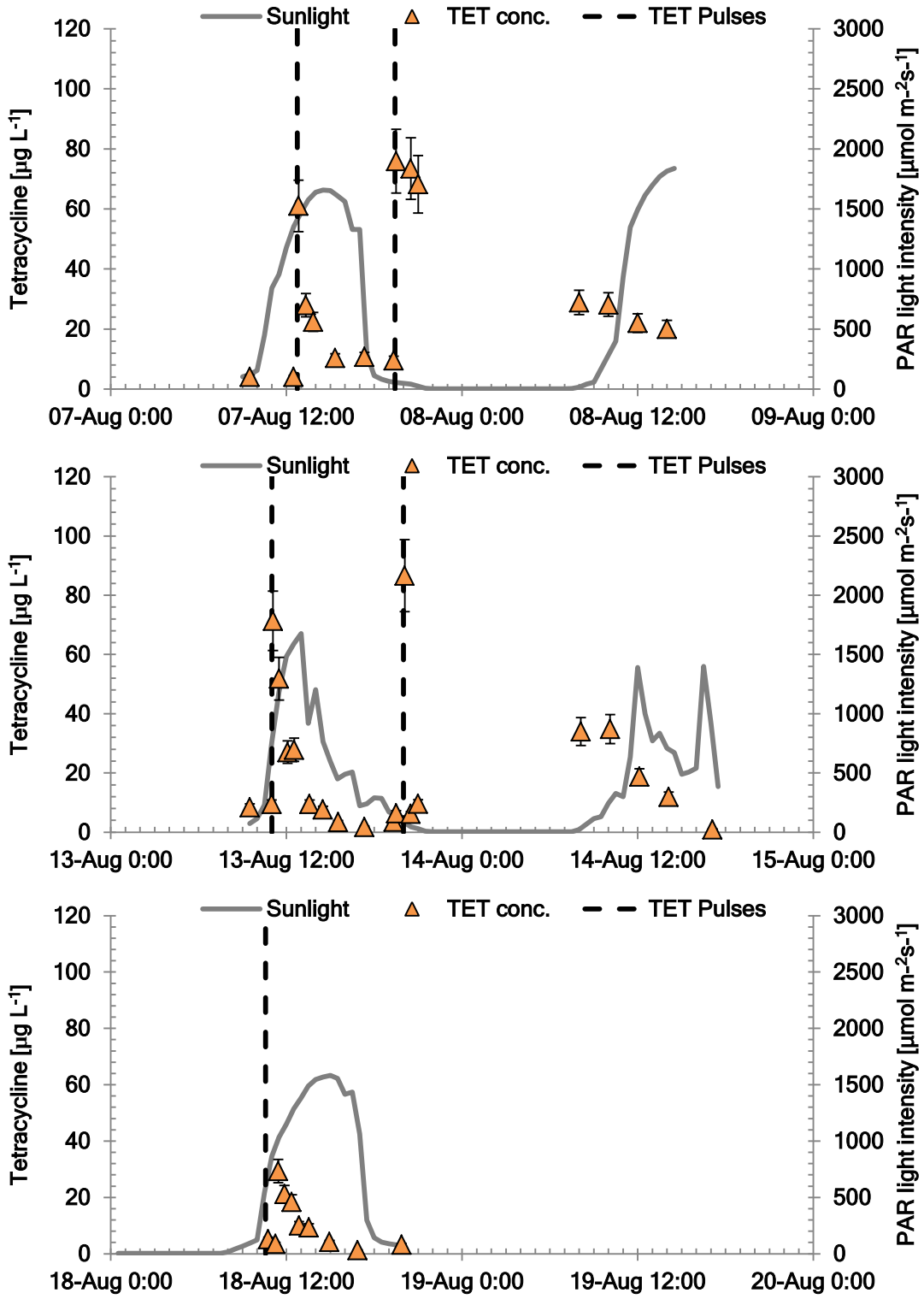

Figure 20: Pulse tests of TET in the 180 L HRAP, August 2015. Sunlight intensity data (continuous line) is shown as half-hour averages. Two TET pulses were added during each test on 7th and 13th of Aug 2015; only one TET pulse was added 18th Aug 2015. Error bars show typical analysis error (see Table 15).

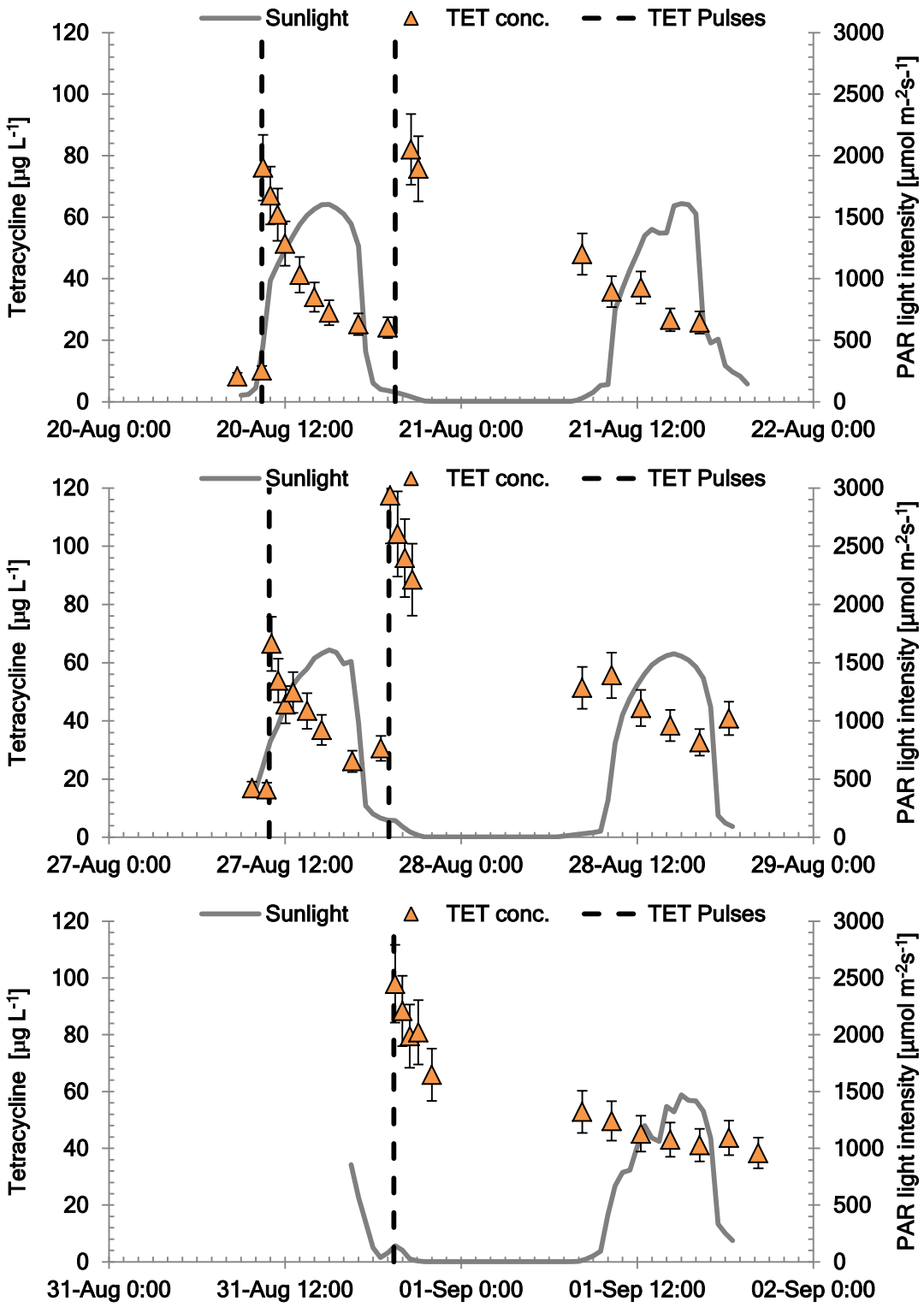

Figure 21: Pulse tests of TET in the 180 L HRAP, August 2015. Sunlight intensity data (continuous line) is shown as half-hour averages. Two TET pulses were added during each test on 20th and 27th of Aug 2015; only one TET pulse was added 31st Aug 2015. Error bars show typical analysis error (see Table 15).

The TET removal results shown in Figure 20 and Figure 21 can be grouped into three different categories, using the terms 'Day 1' and 'Day 2' to refer to data from the first and second days of each TET pulse test :

1. TET removal in the light after TET addition in the morning during Day 1
2. TET removal in the dark (overnight) after TET addition in the evening of Day 1
3. TET removal in the light during Day 2 after TET addition in the evening of Day 1

In order to discuss the differences in TET removal in these three periods more accurately, the pseudo-first order kinetic constants calculated are listed in Table 22. These rates were calculated assuming that the HRAP was a well-mixed system with a constant effluent flow-rate[a].

- During all the pulse tests (Figure 20 and Figure 21), TET removal in the light on Day 1 was rapid (k_1 = 2.4-9.9 d^{-1}; Table 22), with TET concentrations usually stabilising after direct sunlight exposure stopped in the evening. Both sorption and photodegradation likely contributed to the observed TET removal, based on previous results (Sections 4.2 and 4.3). TET desorption may also have occurred due to photodegradation during Day 1, but any TET desorption would have been much less than TET sorption.
- Based on the results from the batch tests (Section 4.3), we expect that sorption was the only mechanism significantly contributing to TET removal during the night following the TET pulse in the evening of Day 1, and TET sorption should be near equilibrium by morning, resulting in a stable[b] aqueous TET

[a] Average effluent flow-rate was 35.6 ± 2.9 L d^{-1} during Stage IV when the pulse tests were performed (Appendix One Section S1.3.2).
[b] The only further TET removal at the end of the night should be due to TET leaving in the effluent, and any biomass growth would allow further TET sorption. However, these removal rates should be slow in

concentration. These predictions were confirmed by the results observed: The decline in TET concentrations is clearest for the pulse tests starting the 27th Aug and the 31st Aug (Figure 21),[a] and in the morning of Day 2 before the sunlight intensity increased the TET concentrations were typically stable (Figure 20 and Figure 21).[b] Overall, the k_1 values for TET removal during the night varied from 0.8 to 1.7 d^{-1} (Table 22).

- As the sunlight intensity increased during Day 2, TET removal also increased slightly (Figure 20 and Figure 21). This increase in TET removal was expected, since TET photodegradation could occur in the light. Because TET sorption should have approached equilibrium during the night, and no further TET was added during Day 2, further TET sorption can only occur due to biomass growth producing more biomass. However, as TET photodegradation reduced the aqueous TET concentration, TET desorption should have occurred.[c] Pseudo-first order TET removal rates in the light during Day 2 were 0.3-6.2 d^{-1} (Table 22). These rates were 1.6 to 3.1 times lower than in the light for Day 1 in the same TET pulse test (k_1 = 2.4-9.9 d^{-1}; Table 22),[d] most likely due to greater TET sorption during Day 1, and greater TET desorption during Day 2.

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comparison to the TET removal by sorption immediately after the TET addition, resulting in comparatively stable aqueous TET concentrations.

[a] Some of the TET concentration data after the evening TET pulse on Day 1 had to be discarded as outliers, which resulted in so only a few data points are seen in the evening of Day 1 for the pulses beginning 13th Aug, and 21st Aug.

[b] Aqueous TET concentrations were not stable for the 21st Aug, but this could be due to analytical uncertainty

[c] Similar to the TET desorption discussed in Section 4.3.1, with the outdoor batch tests. Sorption extraction tests were performed during some of the pulse tests in an attempt to confirm the sorption and desorption occurring. Sorbed TET did increase after the TET pulses, and then decrease during sunlight exposure, but based on a mass balance analysis on sorbed + aqueous TET concentrations, the data from the sorption extraction was considered unreliable (See Appendix One Section S1.4.2 for the mass-balance analysis).

[d] For each pulse test, the TET removal rates achieved at Day 1 and Day 2 were statistically significant ($p<0.1$) except the first pulse test on 7th-8th Aug ($p = 0.12$), possibly due to the smaller number of samples analysed (and therefore greater error.) on Day 2 for the 8th Aug compared to the later pulse tests.

In these pulse tests, both photodegradation and sorption had a major impact upon TET removal. If we consider the theoretical initial TET concentration after the evening TET pulse, TET removal during the night varied from 57% to 74%, with up to 11% of this TET removal due to TET leaving in the effluent.[a] Therefore TET sorption appears to be responsible for 46-63% of the TET removal during the night[b]. This stands in contrast to the 5.3% TET removal by sorption during the Stage III operation at 4-day HRT with TET supplied continuously with the WW. However this difference makes logical sense based on the mechanisms involved. During continuous TET supply during Stage III, sorption should have contributed to removing about 50% of the TET entering the HRAP during the night with the WW influent. However, photodegradation during the day was sufficient to decrease the aqueous and sorbed TET concentrations again before the next night, including the removal of the TET entering the HRAP during daytime with the WW influent.

The TET removal rates for these pulse tests will be discussed further in Section 4.4.3.3, using a model developed to predict TET removal in the HRAP based on a combination of sorption, desorption, and photodegradation mechanisms.

[a] Based on Equation 2 at the beginning of Section 4.4.3, with $k_1 = 0$.
[b] This removal of TET by sorption during the night is similar to the results from the full-day outdoor batch tests in Section 4.3.1, and also to the removal observed for the TET pulse tests in the 900 L HRAP in Section 4.4.3.1 if TET removal by sorption during the night is calculated based upon the theoretical initial TET concentration after the TET pulse was added.

Table 22: Summary of pseudo-first order TET kinetic constants for pulse experiments.

	TET removal in the light, Day 1	**TET removal at night[a]**	**TET removal in the light, Day 2**
	k1 [d^{-1}] ± st.error (R^2, n)	k1 [d^{-1}] ± st.error (R^2, n)	k1 [d^{-1}] ± st.error (R^2, n)
7-Aug	5.6 ± 1.9 (0.70, 6)	1.69 ± 0.04 (1.00, 4)	1.8 ± 0.3 (0.93, 3)
13-Aug	9.9 ± 1.8 (0.82, 9)	1.67 (n= 2)	6.2 ± 0.2 (0.99, 3)
18-Aug	7.4 ± 1.8 (0.72, 8)		
20-Aug	3.2 ± 0.4 (0.90, 9)	0.85 ± 0.05 (0.99, 3)	1.4 ± 0.3 (0.79, 4)
27-Aug	2.4 ± 0.4 (0.83, 8)	1.15 ± 0.16 (0.95, 5)	0.9 ± 0.4 (0.56, 5)
31-Aug		0.8 ± 0.3 (0.80, 5)	0.3 ± 0.1 (0.78, 6)

[a] The k_1 values for TET removal at night are uncertain, as they do not take into account the initial rapid TET sorption during the 5 min initial mixing, and no data was collected from 10 pm-7 am.

Finally, some observations were made regarding the impact of the TET shock loads upon HRAP performance. In contrast to the pulse tests conducted in the 900 L pilot HRAP, the TET pulse experiments conducted in the 180 L HRAP appeared to stress the microbes in the HRAP, resulting in decreased settleability of the biomass and greater difficulty filtering HRAP samples in Stage IV than in Stage III[a] . The most evident quantification of the change in settleability was a reduction in biomass density in the clarifier from ~15 g L^{-1} in Stage III to ~ 5 g L^{-1} after the final pulse test. Visually, more filamentous biomass was observed in the HRAP at the end of Stage IV than in Stage III. Similarly, de Godos et al. (2012) noted de-flocculation of the algal biomass when TET influent was begun in their lab-HRAP experiments. This change to biomass flocculation/settleability may have had an effect upon TET sorption on biomass (changing the properties of the biomass), or photodegradation (by affecting light attenuation through the water in the HRAP), although these effects were not confirmed.

[a] The increase in difficulty filtering HRAP samples was first noted 1 week after the first TET pulse test. Curiously, the filtering improved for HRAP samples taken during the last TET pulse test performed 31st Aug 2015, despite a continued decrease in the settleability of the HRAP biomass in the clarifier.

4.4.3.3 Trends observed between TET removal, environmental variables, and HRAP wastewater characteristics

To investigate the variation in the TET removal rates, pseudo-first-order TET removal rates (k_1) were plotted against environmental variables (light, pH, DO, temperature). Both 'instantaneous' TET removal rates[a] (Figure 22) and the average TET removal rates[b] (Figure 23) were compared to the averaged environmental variables in the corresponding time period to search for correlations and interesting effects that might explain the variation in TET removal rates. The average TET removal rates were also compared to the WW characteristics in the HRAP. The nitrite, nitrate, and TSS concentrations had the highest correlations with the average TET removal rates, therefore these effect plots are also shown in Figure 23.

Figure 22 shows that the highest TET removal rates observed are all associated with the highest quartile range of DO concentrations, pH, and PAR. The clearest effects can be seen for DO, followed by pH, and then PAR. The potential effects of DO, pH, and PAR are all confounded, as pH and DO depend upon the amount of light received. However, based on literature and previous results, we can suggest reasons for the trends observed:

- Dissolved oxygen concentrations may affect the production of reactive oxygen species (Sandvik et al., 2000; Vaughan and Blough, 1998) involved in the indirect photodegradation of TET.
- pH affects the ionic state of TET (pKa ~7.8, Section 4.3.3), and several studies have reported that direct TET photolysis increases at increased pH, although whether indirect TET photodegradation also increases at increased pH has not

[a] 'Instantaneous' TET removal rates were calculated based on regression of every 4 adjacent data points recorded for the TET concentrations during the pulse tests, see Appendix One Section S1.4.3
[b] 'average' TET removal rates compared are from the TET removal rates during light exposure from Table 22

been confirmed (Chen et al., 2008; López-Peñalver et al., 2010; Niu et al., 2013; Wammer et al., 2011). Increased TET removal at increased pH was also observed in batch experiments discussed in Section 4.3.3, although the mechanism was not identified.

- Increased light intensity should increase photodegradation if the mechanism is not rate-limited elsewhere (e.g. indirect photodegradation may be rate-limited by the generation of reactive oxygen species).

No trends were observed between temperature and the 'instantaneous' k_1 (Figure 22), which suggests that temperature changes do not have any significant direct effect on TET removal rates by improving thermodynamics of the reactions. However, there was a strong correlation ($r = -0.72$, $p = 0.02$) between average temperature and the average daily TET removal (Figure 23). Possible reasons for this include: 1) decreased TET sorption at higher temperatures (see Section 2.3); and 2) stress caused to the algal-bacterial biomass at higher temperatures causing indirect effects on TET sorption and photodegradation.

The low nitrite concentrations were correlated with low TET removal (Figure 23; $r = 0.6$, $p = 0.07$), which is similar to the decrease in TET removal seen in the 900 L HRAP (Section 4.4.1) after a drop in nitrite concentrations. As discussed in Section 4.4.1, nitrifiers can biodegrade TET under some conditions, and this could explain the correlation. There is however insufficient evidence to prove a causal relationship.

The TSS concentration was highly correlated with the average daily TET removal (Figure 23; correlation = 0.83), which could be related to TET sorption. The correlation was statistically significant ($p < 0.01$), however this correlation was heavily influenced

by the two data points with TSS concentration >1.3 g L^{-1}, and may simply be due to inaccurate measurement of TSS concentration.

As shown in Figure 23, although the first order kinetics rates of TET removal were statistically correlated ($p \leq 0.1$) to total PAR received, pH, average temperature, TSS concentration, nitrite concentration, and nitrate concentration, these correlations were insufficient to confirm any causal relationships due to the confounding between variables.

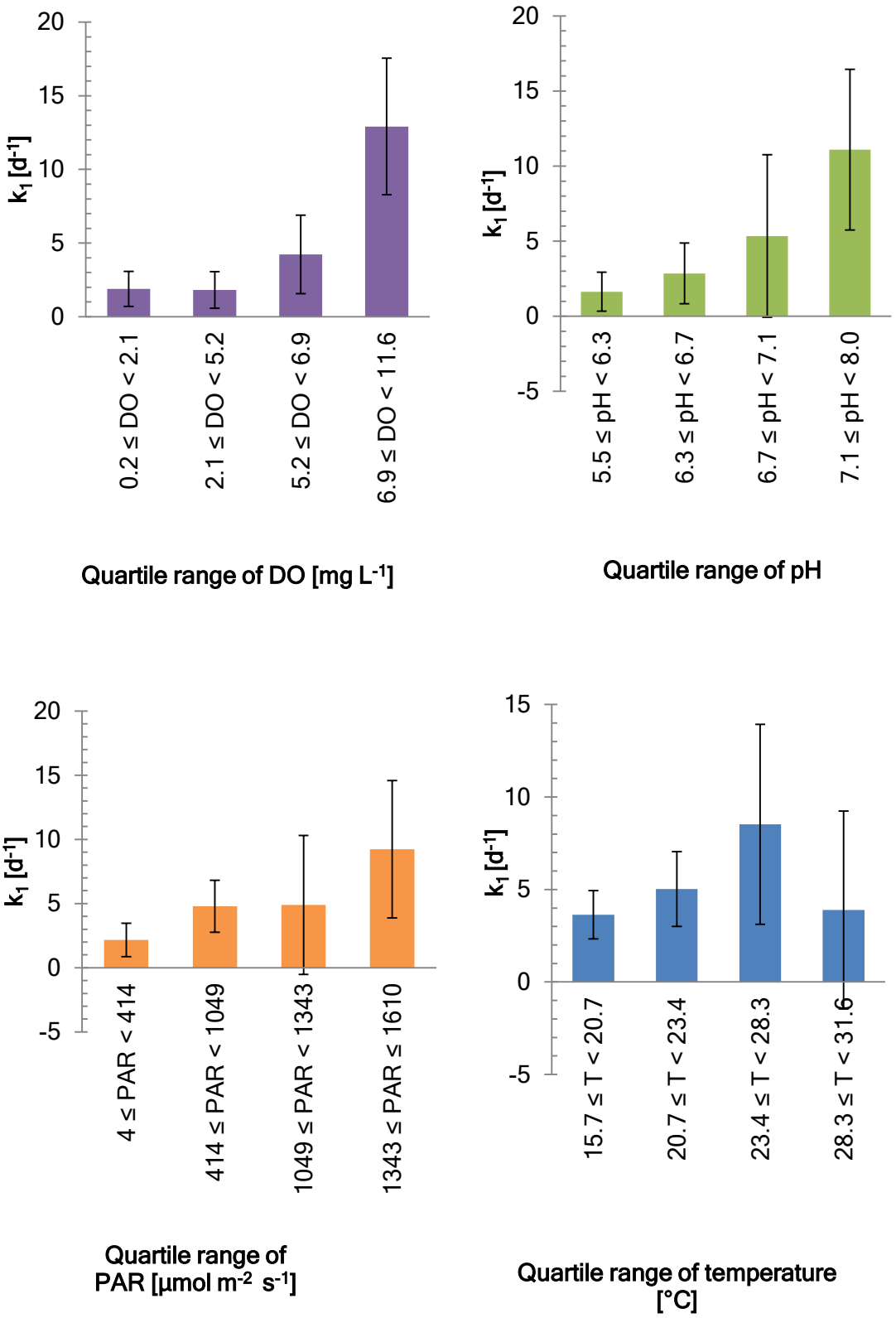

Figure 22: The quartile ranges of the pH, DO concentration, PAR, and temperature plotted against the pseudo-first-order TET removal rates (k_1) in each range. Error bars show the standard deviation of the TET removal rates in each quartile range (n=13).

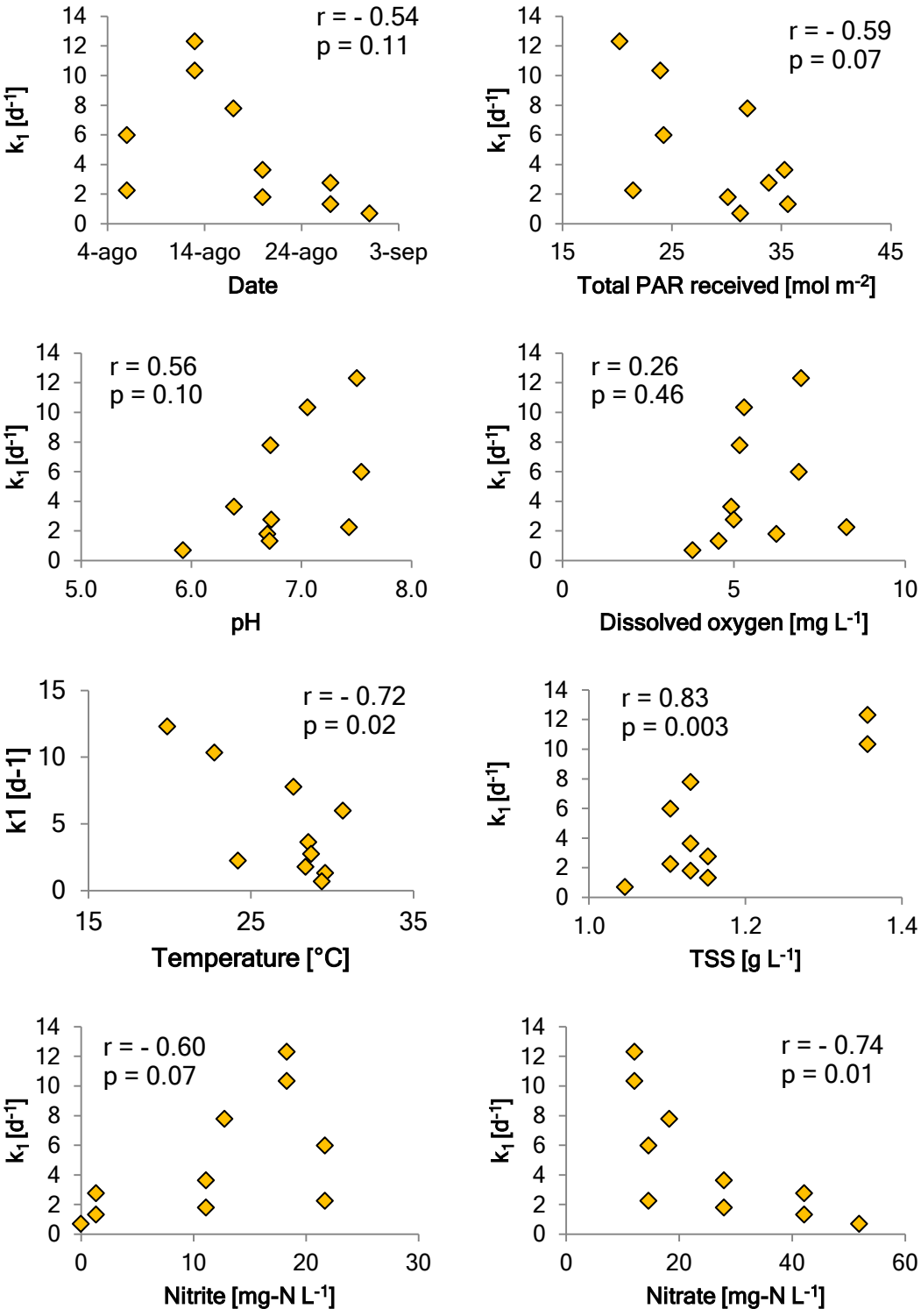

Figure 23: Effects plots for the average daily first-order TET removal rates (k_1) during the day vs. the date, total light received, pH, DO concentration, temperature, TSS concentration, nitrate, and nitrite concentrations in order to visualise the trends. Each graph includes the Pearson's correlation coefficient (r) and the p-value (p).

4.5 Prediction of TET removal compared with experimental data

This section presents the equations developed to predict TET removal in HRAPs. These equations were used to predict TET removal during the TET pulse tests in the 180 L pilot HRAP, and compared with the experimental data. The kinetic constants and sorption partition coefficients required to parameterize the equations were obtained from experimental results independent of the TET pulse tests: the batch experiments (Section 4.3), and the sorption partition coefficients measured during Stage III of the 180 L pilot HRAP operation (Section 4.4.2). The purpose of these predictive equations was to compare the batch tests and the pilot HRAP experimental data, taking into account the differences in experimental design and conditions. Furthermore, if equations based on the kinetic data from the batch tests and the design and operational parameters of the pilot HRAP indeed provide an accurate prediction of the pilot HRAP TET pulse results, these equations could be used to predict TET removal for HRAP systems with different designs and operating conditions.

The following assumptions were made to simplify predictions:

- The HRAP was completely mixed.
- TSS concentrations and were constant during modelling.[a]
- Biomass productivity could be modelled as a constant rate.
- Differences in environmental conditions between experiments (pH, DO concentration, and temperature) did not affect TET removal.[b]
- Photodegradation only affected aqueous TET, and did not affect sorbed TET.

[a] There was insufficient data to model the effect of TSS concentrations on TET removal by photodegradation, and for TET removal by sorption the effect of fluctuating TSS concentrations was minimal compared to other uncertainties. In a 'worst case' scenario where the biomass does not leave in the effluent, but accumulates in the HRAP, the change in TSS concentration over 24 hours is <10% (based on net average biomass productivity of 18 g d^{-1}, average TSS concentration during Stage IV of 1.2 g L^{-1}, and 180 L HRAP volume).

[b] We do not have enough experimental data to develop equations that can predict a causal relationship between the environmental conditions and the TET removal rates.

4.5.1 TET removal by sorption during the night

This section presents equations developed to describe TET removal by sorption, in order to predict TET removal during the night after TET pulse addition. Without light exposure, photodegradation is irrelevant, so the only relevant TET removal should occur by TET sorption or effluent flow. Batch tests (Section 4.3.2) indicated that sorption should be near equilibrium after 14 h. If we assume that TET sorption was also near equilibrium immediately before the TET addition, then TET removal by sorption overnight can be predicted by mass balance using the equilibrium partition coefficient K_d for TET sorption to the HRAP biomass:

$$C_0 * V + q_0 * TSS * V + TET_{added} = C_f * V + q_f * TSS * V - Loss_{Effluent} \quad (equation\ 3)$$

$$and \quad q_{eq} = K_d * C_{eq} \quad (equation\ 4)$$

Where C is the aqueous TET concentration (µg L^{-1}) and q is the sorbed concentration of TET (µg g^{-1}), 'TET_{added}' is the amount of TET added as a pulse (µg), V is the volume of the HRAP (L), 'TSS' is the TSS concentration (g L^{-1}), K_d is the sorption partition coefficient at equilibrium (L g^{-1}), and '$Loss_{Effluent}$' is the amount of TET that leaves the HRAP during the night in the effluent (µg). The subscripts of 'C' and 'q' ('0', 'f', and 'eq') refer to concentrations immediately before TET addition, concentrations the next morning (>14 h after TET addition), and concentrations at equilibrium, respectively. Based on our assumptions, $q_0 \approx K_d \times C_0$ and $q_f \approx K_d \times C_f$.

1. In order to quantify the amount of TET leaving the HRAP ('$Loss_{effluent}$'), the total aqueous and sorbed TET concentration ('TOTAL', µg L^{-1}) can be represented by Equation 5:

$$TOTAL = C + q * TSS \quad (equation\ 5)$$

The total TET removed in the effluent from immediately after the TET addition to the next morning can then be integrated over time using standard equations for a continuous well-mixed system with no influent TET concentration:

$$TOTAL_f = \left(TOTAL_0 + \frac{TET_{added}}{V}\right) * e^{\left(-\frac{Q_{out}}{V}*(t_f - t_0)\right)} \quad (equation\ 6)$$

$$and\ Loss_{effluent} = \left(TOTAL_0 + \frac{TET_{added}}{V}\right) - TOTAL_f \quad (equation\ 7)$$

Where Q_{out} is the effluent flow-rate (L d^{-1}), and t is the time (d). The other variables and subscripts are defined above.

Three different values of equilibrium partition coefficients were available:

- the K_d value calculated from the batch tests results in Section 4.3.2 was K_d = 1.4 ± 0.1 L g^{-1} (std. error of regression, n=3)
- the individual K_d values calculated during Stage III (4 day HRT) of the 180 L pilot HRAP were K_d = 4.6 L g^{-1} and K_d = 2.8 L g^{-1} for the two analyses of sorbed TET concentrations (Section 4.4.2).

The three K_d values listed above were used for calculating the predicted TET removal via sorption using Equations 3 to 7: a low estimate (K_d = 1.4 L g^{-1}), a mid-range estimate (K_d = 2.8 L g^{-1}), and a high-range estimate (K_d = 4.6 L g^{-1}). Figure 24 presents the comparison between observed and predicted aqueous TET concentrations at time = t_f (the morning after the TET addition). In three of the five cases, observed values fell within the error bounds of the predicted values, but overall the predictions predicted greater TET removal during the night than was observed.

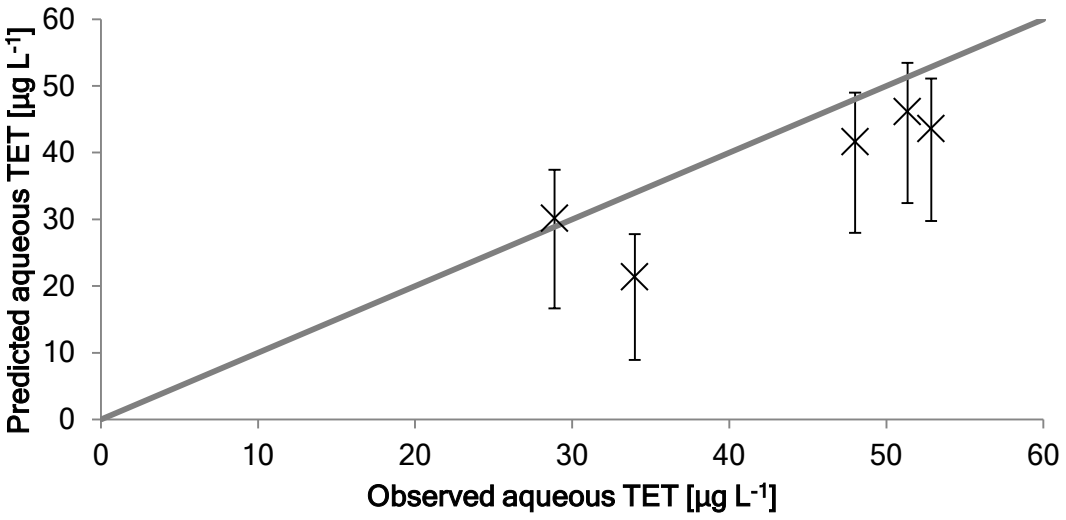

Figure 24: Predicted vs. observed aqueous TET concentrations in the morning of 'Day 2' for the pulse tests, based on a mid-range estimate of K_d = 2.8 L g^{-1}. The error bars show the range of predictions from K_d = 1.4 to 4.6 L g^{-1}. The diagonal grey line represents equality between observed and predicted values.

The two most likely explanations for the over-prediction of TET removal are:

- The assumption that the HRAP was well-mixed over-estimates the TSS concentration in the effluent compared with measured values, and thus over-estimates the amount of sorbed TET leaving in the effluent. To solve this problem the amount of sorbed TET leaving in the effluent must be modelled based on sorbed TET concentrations and the measured biomass productivity, rather than the average TSS concentration in the HRAP.
- Four out of the five TET additions at night occurred after an earlier TET pulse added that morning. Therefore TET sorption may not have been near equilibrium immediately before the TET addition at night, as assumed for the predictions in this section. To solve this problem the sorbed TET concentrations during the earlier TET pulse must be modelled.

Therefore to improve the modelling of the TET pulses, and to mitigate the impact of the two problems identified above, a kinetic model was developed and is presented in the following section (4.5.2).

4.5.2 Kinetic modelling of sorption and photodegradation

This section presents the developed kinetic equations for TET removal by sorption and photodegradation, and then the combined predictive model for TET removal along with comparison between model predictions and experimental data.

4.5.2.1 Sorption kinetic equations

Although sorption contributed little to TET removal during stable HRAP operation with continuous TET supplied in the influent WW, this mechanism could have a large impact in mitigating any sudden increases in TET supply, or mitigating fluctuating TET concentrations between sunlight hours and night-time. Sorption could also have a greater impact on TET removal during winter months, when light supply is low. In order to model sorbed TET concentrations during unsteady-state conditions, sorption and desorption kinetic rates both needed to be determined. In Section 4.3.2, a pseudo-first-order kinetic rate provided the best fit to TET sorption (k_{1s} = 15 ± 1 d^{-1}), based on Equation 1, repeated here:

$$\frac{dC}{dt} = k_{1s} * C - k_{1ds} * q * TSS \qquad (equation\ 1)$$

Where dC/dt is the rate of aqueous TET removal by sorption, k_{1s} and k_{1ds} are the pseudo-first-order kinetic constants for sorption and desorption respectively (d^{-1}), C is the aqueous TET concentration (mg L^{-1}), q is the sorbed TET concentration (mg g^{-1}), and 'TSS' is the TSS concentration (g L^{-1}) of the HRAP.

Desorption kinetics are difficult to determine (Limousin et al., 2007), but since the sorption equilibrium (K_d) can be described as the point when the rates of sorption and desorption are equal (equation 4, $q_{eq} = K_d * C_{eq}$), the following equation can be derived:

$$k_{1s} * C_{eq} = k_{1ds} * q_{eq} * TSS_{eq} \qquad (equation\ 8)$$

As TSS is approximately constant,[a] the first-order desorption kinetic constants can thus be estimated as:

$$k_{1ds} = \frac{k_{1s}}{K_d * TSS}; \qquad (equation\ 9)$$

For example, using a K_d value of 2.8 g L^{-1} and the first-order kinetic rate estimated from the sorption batch experiment (k_{1s} = 15 ± 1 d^{-1}), the desorption kinetic rate was thus estimated as k_{1ds} = 4.5 d^{-1} at TSS = 1.2 g L^{-1}.

To quantify the rate of sorbed TET leaving in the effluent, we need to account for biomass productivity:

$$\frac{dq}{dt} * TSS * V = -q * P_{biomass} \qquad (equation\ 10)$$

Where dq/dt is the rate of change of the sorbed TET concentration (mg g^{-1} d^{-1}), 'TSS' is the TSS concentration (g L^{-1}), V is the volume of the HRAP (L), q is the sorbed TET concentration (mg g_{TSS}^{-1}), and $P_{biomass}$ is the biomass productivity (g d^{-1}).

As TSS was a constant for the purposes of the kinetic model, this Equation 8 assumes that there is an equal rate of biomass growth maintaining the TSS concentration in the HRAP, equal to the rate of biomass leaving in the effluent ($P_{biomass}$).

Equation 8 can be simplified using the sludge retention time, SRT (d), which is equal to the total mass of biomass in the HRAP divided by the biomass productivity:

$$SRT = \frac{TSS * V}{P_{biomass}} \quad (equation\ 11), \qquad therefore \quad \frac{dq}{dt} = -\frac{q}{SRT} \quad (equation\ 12)$$

[a] This assumption was made and justified at the beginning of Section 4.5

4.5.2.2 Photodegradation kinetic equations

Photodegradation kinetics are frequently described in terms of a quantum yield[a] (φ, mol/mol) based on the light energy received:

$$V * \frac{dN}{dt} = \varphi * I_{av} * A \qquad (equation\ 13)$$

Where V is the volume (L), dN/dt is change in the molar TET concentration over time (mol L^{-1} s^{-1}), I_{av} is the average light intensity through the system (mol m^{-2} s^{-1}), and A is the surface area (m^2).

Two adaptations were made to this standard equation:

- Based on previous results (Sections 4.2 and 4.3.1), we have observed that the photodegradation appears to have a pseudo-first order dependence upon TET concentration. The quantum yield in Equation 11 was therefore replaced by the TET concentration multiplied by a term we have called the 'photodegradation yield' (k_{1L}, L µmol^{-1}).
- Average light intensity (I_{av}) is normally calculated by integrating the light intensity at any depth over the depth of the system, based on an extinction coefficient that describes light attenuation through the water. However, we did not have enough data to estimate an appropriate extinction coefficient. Therefore, to remove the necessity to calculate I_{av}, we assumed:
    - The system is sufficiently deep to fully attenuate (absorb) all the light entering the system, and thus the average light intensity (I_{av}) is proportional to the surface PAR light intensity (I_0) if light attenuation is constant.

[a] When light supply limits photodegradation kinetics (i.e. the specific reaction of photon with TET can be considered as near instantaneous and therefore, at equilibrium with regards to the rate of photon supply to the culture), the time-independent quantum yield (an equilibrium constant) dictates the kinetics rates.

The proportional difference between I_{av} and I_0 was therefore included in our term the 'photodegradation yield' (k_{1L}), which makes k_{1L} dependent upon light attenuation. Thus if the same 'photodegradation yield' (k_{1L}) in Equation 12 is used for two different systems, it is assumed that light attenuation is the same in both systems.

Thus Equation 14 describes the TET photodegradation kinetics, with TET concentration (C) expressed as mg L^{-1} and PAR surface light intensity (I_0) expressed as µmol m^{-2} d^{-1}:

$$V * \frac{dC}{dt} = k_{1L} * C * I_0 * A \qquad (equation\ 14)$$

Where V is the volume (L), dC/dt is change in the TET concentration over time (mg L^{-1} d^{-1}), k_{1L} is the 'photodegradation yield' (L µmol^{-1}), C is the TET concentration (mg L^{-1}), I_0 is the surface PAR light intensity (µmol m^{-2} d^{-1}), and A is the surface area (m^2).

4.5.2.3 Full predictive kinetic model

This section presents the full kinetic model based on the equations developed in the previous sections, compares model predictions of TET concentrations during the pulse tests in the 180 L HRAP to observed TET concentrations based on kinetics determined from independent experiments, and also compares model predictions to the observed TET concentrations during Stage III operation of the 180 L pilot HRAP.

The change in aqueous TET concentration in the pilot HRAP (dC/dt) is the sum of the rate of TET entering the HRAP, the rate of TET leaving the HRAP, the rate of TET removed by photodegradation, the rate of TET removed by sorption, and the rate of TET desorption (see Table 23 for the list of variable definitions and units):

$$\frac{dC}{dt} = \frac{C_{in} * Q_{in} - C * Q_{out}}{V} - \frac{k_{1L} * C * I_0 * A}{V} - k_{1s} * C + k_{1ds} * q * TSS \qquad (equation\ 15)$$

The change in sorbed TET concentration is the sum of the rate of TET sorption, the rate of TET desorption, and rate of TET leaving with the biomass in the effluent (see Table 23 for the list of variable definitions and units):

$$\frac{dq}{dt} = k_{1s} * \frac{C}{TSS} - k_{1ds} * q - \frac{q}{SRT} \qquad (equation\ 16)$$

Table 23: Variables used in the TET removal kinetic model (Equations 15 & 16)

Variable	Description	Units
dC/dt	rate of change of aqueous TET concentration	mg L^{-1} d^{-1}
C	aqueous TET concentration	mg L^{-1}
dq/dt	rate of change of the sorbed TET concentration	mg g^{-1} d^{-1}
q	concentration of TET sorbed to the algal-bacterial biomass	mg g^{-1}
C_{in}	TET concentration in the influent	mg L^{-1}
k_{1L}	pseudo-first-order 'photodegradation yield'	L µmol^{-1}
k_{1s}, k_{1ds}	pseudo-first-order sorption and desorption kinetic constants	d^{-1}
V	volume of the HRAP	L
A	surface area of the HRAP	m^2
Q_{in}, Q_{out}	influent and effluent flow rate, respectively	L d^{-1}
'TSS'	TSS concentration in the HRAP	g L^{-1}
I_0	surface PAR light intensity	µmol m^{-2} d^{-1}
'SRT'	sludge retention time	d

The sorption kinetic constant was estimated based on the sorption batch test (Section 4.3.2) and desorption constants were calculated based on Equation 9 (Section 4.5.2.1):

$$k_{1ds} = \frac{k_{1s}}{K_d * TSS} \qquad (equation\ 9)$$

The three estimates for the partition coefficient (low, high, and mid-range) used to calculate the desorption constant for predictions were the same as in Section 4.5.1 (Table 24). Three estimates for the photodegradation yield (k_{1L}) were also obtained based on results from three of the full-day outdoor batch experiments discussed in Section 4.3.1. The kinetic model was numerically solved using Matlab R2012b, and the predicted aqueous TET concentrations were manually fitted to the experimental data, adjusting the constants 'k_{1L}' and 'K_d' to minimise and randomly distribute the residuals (R^2 = 0.96-0.99, graphs shown in Appendix Five Section S5.1). The 'k_{1L}' values thus obtained for the 100% active HRAP biomass were 4.2 ×10^{-5}, 2.1 ×10^{-5}, and 6.0 ×10^{-5} L µmol^{-1} for the 28th May, 1st June, and 5th Aug respectively. These three 'k_{1L}' values were thus used for low, high, and mid-range estimates for further predictions, as shown in Table 24. The first-order sorption kinetic constant was held constant at k_{1s} = 15 d^{-1}.

Table 24: Constants used to predict TET concentrations in the 180 L pilot HRAP

Constants	**Average estimate**	**High estimate**	**Low estimate**
k_{1L} [L µmol^{-1}]	4.2 ×10^{-5}	6.0 ×10^{-5}	2.1 ×10^{-5}
K_d [L g^{-1}]	2.8	4.6	1.4

For predicting TET removal in the 180 L HRAP during the pulse tests, average values for Q_{in} (43.6 L d^{-1}), and Q_{out} (35.6 L d^{-1}) were used (Appendix One, Section S1.3.2). Data for I_0 was taken from the light meter data-logging. Surface area was 1.2 m^2. SRT was set at 11 days, based on the average productivity of the HRAP (19.2 g m^{-2} d^{-1}), the

average TSS concentration (1.2 g L^{-1}), and the HRAP volume. The model was numerically solved with Matlab R2012b, using the constants shown in Table 24.

An example of the predicted TET concentrations is shown in Figure 25, with the other graphs included in Appendix Five Section S5.2. Figure 25 shows that the kinetic model can approximately predict the observed aqueous TET concentrations, with most of the observed aqueous TET concentrations between the 'low' and 'high' predicted aqueous TET concentrations. The model predictions shown in Figure 25 also show how the photodegradation of aqueous TET during day time triggers a reduction of sorbed TET concentration due to TET desorption from the algal biomass (i.e. as aqueous TET concentration decreases, TET desorption must take place to achieve a new sorption equilibrium, see Section 4.4.3.2).

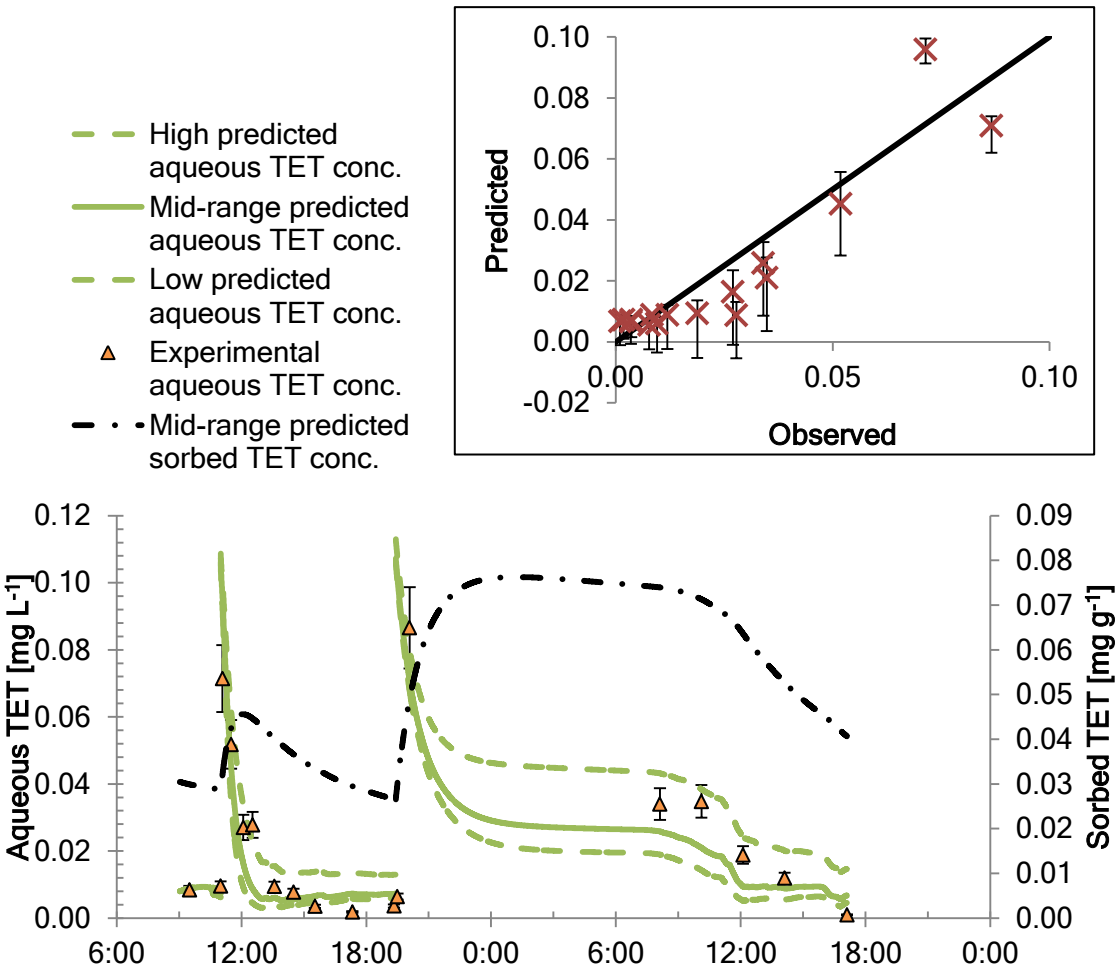

Figure 25: Predicted TET concentrations with high/low error bounds compared to experimental TET concentrations for the pulse test performed 13th Aug 2015. Error bars show typical analysis error (see Table 15). Inset: predicted vs. observed aqueous TET concentrations, with error bars showing the range of predicted values using the 'low' to 'high' estimated K_d and k_{1L} values.

However, of the six pulse tests performed, the kinetic model predictions based on the independent values for k_{1L} and K_d only accurately predicted the first three pulse tests (7th, 13th, and 18th Aug).[a] Figure 26 shows this trend, with predicted aqueous TET concentrations consistently lower (i.e. greater TET removal) than the observed aqueous

[a] Observed TET removal for the pulse test on the 18th Aug did fall within the predicted TET removal estimates, but the validation for this pulse test is uncertain due to the large initial TET removal (only 27 µg L^{-1} increase after the pulse, Section 4.4.3.2).

TET concentrations during pulse tests on the 20th, 27th, and 31st Aug 2015. We were not able to identify any specific change that caused this reduction in TET removal. The most likely causes include changes to pH or DO concentrations as discussed in Section 4.4.3, or changes to light attenuation related to the decreased settleability of the HRAP biomass (see Section 4.4.3.2).

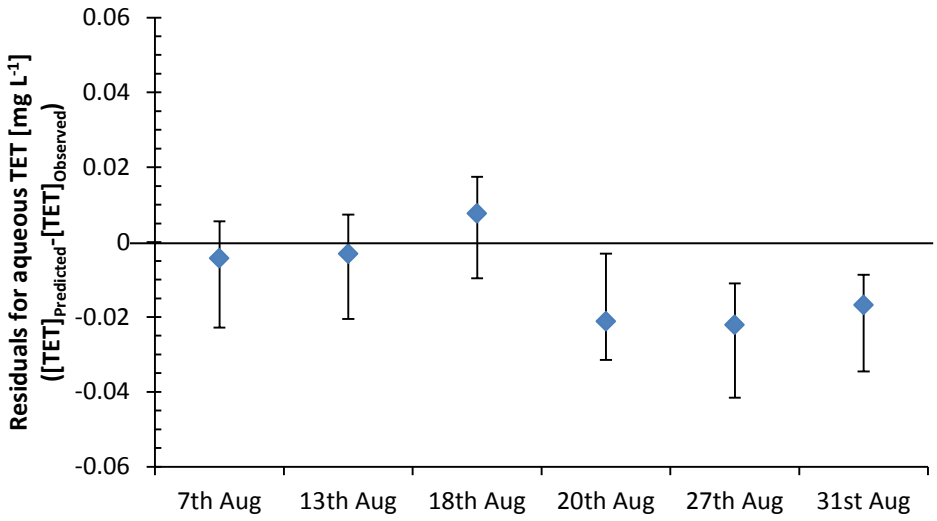

Figure 26: Average residuals for the 'mid-range' predicted aqueous TET concentrations vs. observed aqueous TET concentrations, for each pulse test in the 180 L HRAP. Error bars show the average difference from 'mid-range' predictions to 'low' or 'high' predictions.

This kinetic model was also used to predict TET removal during HRAP operation with continuous TET supplied in the influent WW, as in Stages II and III for the 180 L HRAP. TET removal during Stage III operation of the 180 L HRAP was thus predicted using the kinetic model, again using the 'high', 'mid-range', and 'low' parameter estimates. Average flow-rates for Q_{in} and Q_{out} were 42.9 L d^{-1} and 33.9 L d^{-1} respectively, average TSS concentration was 1.3 g L^{-1}, and SRT was approximately 13

days, based on a biomass productivity of 18 g L^{-1} (Appendix One, Section S1.3.2). Sunlight was not data-logged during Stage III operation, so daily I_0 data was taken from data recorded on the 14th Aug (a cloudy day, in order to have a conservative estimate of TET removal). The model was run for 30 days to obtain stable TET concentrations.

The results of these predictions are listed in Table 25. The observed aqueous TET concentrations during the same period were 5.2 ± 0.9 µg L^{-1} (mean ± 95%CI, n=8) in the morning and 2.5 ± 0.5 µg L^{-1} (mean ± 95%CI, n=6) in the evening (Section 4.4.2), which should correspond to the maximum and minimum predicted values, respectively. Observed sorbed TET concentrations were 12.3-12.6 µg g^{-1}.[a] Indeed, the observed aqueous TET concentrations were similar to predicted concentrations for both the 'mid-range' and 'high' estimated parameters. The model predicted a greater daily fluctuation in the aqueous TET concentration than was observed, possibly due to analytical uncertainty or unknown mechanisms mitigating extreme values during HRAP operation.

No more experimental data was available to adapt the kinetic model further to consider other variables (e.g. light attenuation, pH, DO concentration). Accounting for these factors may explain the decrease in TET removal observed for the final three TET pulse tests. Since further development of the model was not practical based on available experimental data, the lower TET removal during the final three TET pulse tests was quantified by fitting the k_{1L} and K_d values to the experimental data (Section 4.6).

[a] For these predictions, only the 'low estimate' K_d value was independent of the experimental data, as the other two K_d values used as estimates were obtained during Stage III operation.

Table 25: Predicted aqueous and sorbed TET concentrations during continuous TET supply and 4 day HRT operation in the 180 L pilot HRAP.

	Mid-range estimate	High estimate	Low estimate
k_{1L} [L µmol^{-1}]	4.2×10^{-5}	6.0×10^{-5}	2.1×10^{-5}
K_d [L g^{-1}]	2.8	4.6	1.4
	Predicted aqueous TET concentrations (C, [µg L^{-1}])		
Max daily [C]	6.9	5.7	10.4
Average daily [C]	4.9	3.9	7.9
Min daily [C]	2.1	1.4	4.6
	Predicted sorbed TET concentrations (q, [µg g^{-1}])		
Max daily [q]	13.4	17.4	11
Average daily [q]	16.7	21	13.8
Min daily [q]	10.4	14.3	8.4

Without a more suitable model to test, the kinetic model developed in this section should therefore serve as a preliminary model of TET removal in HRAPs. The model was partially validated based on its accurate estimation of TET removal during the first three pulse tests and the TET removal during Stage III operation, but over-predicted TET removal after HRAP conditions changed between the 15th and 20th Aug 2015.

4.6 Comparison between TET removal across all batch tests and pulse tests in the pilot HRAPs

In this section, the kinetic model was used to quantify the variation of TET removal in the light and dark periods, by adjusting the 'photodegradation yield' (k_{1L}) and the sorption partition coefficient (K_d) to fit the predicted TET concentrations to the experimental data. These fitted constants will provide a means to compare the experimental data in the batch tests and the pulse tests in the two pilot HRAPs (180 L and 900 L) while accounting for the main differences in operation and design. The fitted constants will also provide a more conservative estimate of TET removal based on the lower TET removal observed during the last three pulse tests in the 180 L HRAP.

The 'photodegradation yield' (k_{1L}) and the sorption partition coefficient (K_d) were manually fit to the experimentally measured TET concentrations, to obtain a roughly random spread of residual values (see Figure 24).

In Figure 27, an example is provided of the kinetic model fitted to the experimental data. The best fit values of the kinetic parameters for all the pulse tests are listed in Table 26.[a] The contribution of sorption to the overall removal of TET during the pulse tests is predicted to be about 50%,[b] as discussed in Section 4.4.3.2 , which is seen visually in Figure 27. If no further TET pulses occur, photodegradation should continue to reduce both the aqueous and the sorbed TET concentrations during daytime. These predicted sorbed TET concentrations were not validated, due to uncertainties in the data

[a] see Appendix Seven Section S7.2 for all the graphs. For the 900 L HRAP, Q_{in} was 100 L d^{-1}, and Q_{out} was 90 L d^{-1} (estimated based on the open-water evaporation data for Palmerston North, see Section 4.4.3.1). Average TSS concentrations were used (0.6 g L^{-1}). Surface area was 3.5 m^2. Productivity and SRT were calculated based on average TSS concentrations and Q_{out}, with a resulting productivity of 54 g_{TSS} d^{-1}, and an SRT of 10 days. The design and operation parameters for the 180 L HRAP used in the kinetic model were described in Section 4.5.2.

[b] based on the ~1.1 g L^{-1} TSS concentration in the 180 L pilot HRAP

analysis for the sorbed TET concentrations measured during the pulse tests.[a] Normally, we would not expect large TET pulses to occur in WW, and therefore the contribution of sorption to overall removal should be more similar to the sorption observed during continuous TET supply (e.g. ~5% for the 4-day HRT operation in the 180 L HRAP, see Section 4.4.2).

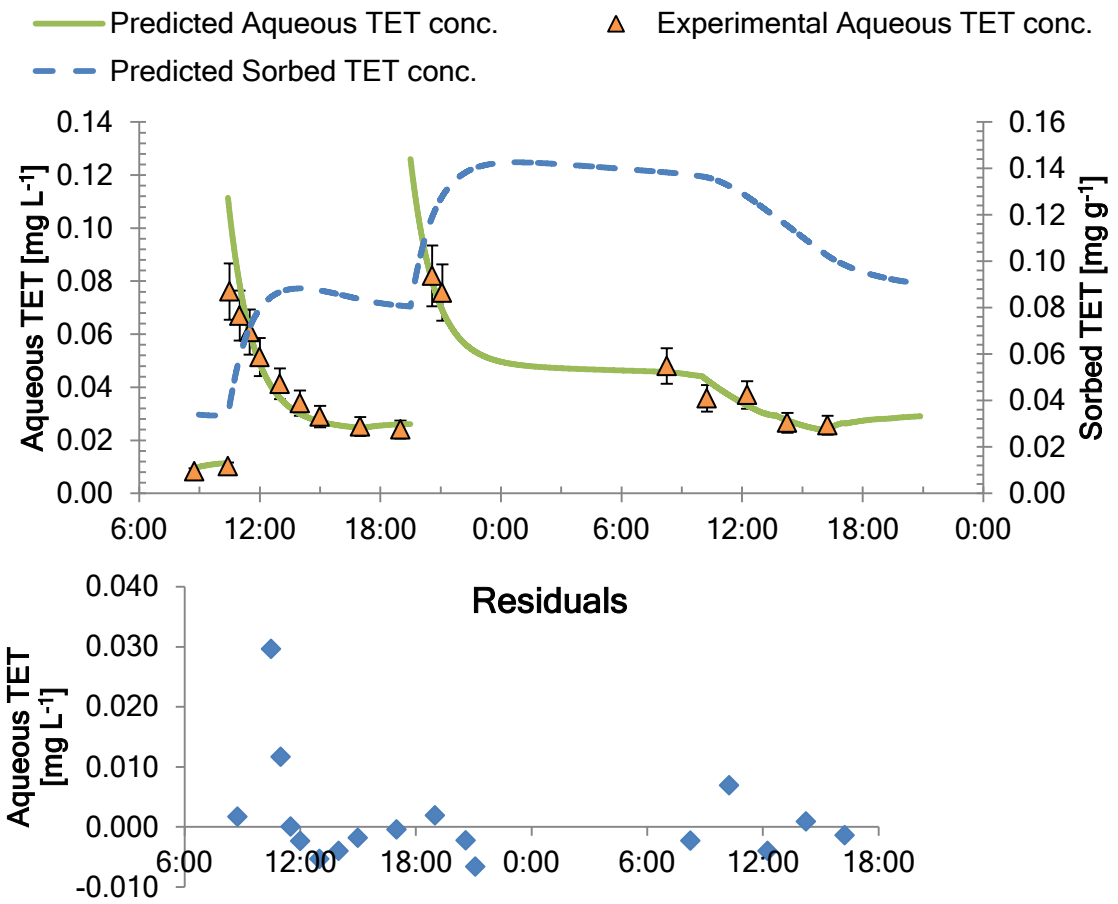

Figure 27: Modelled aqueous and sorbed TET concentrations for the pulse test starting 20th Aug 2015, based on best-fit photodegradation kinetics and sorption partition coefficient (see Table 26) compared with the experimental aqueous TET concentrations. Error bars show typical analysis error (see Table 15).

[a] Sorption extraction tests were performed during some of the pulse tests in an attempt to confirm the sorption and desorption occurring. Sorbed TET did increase after the TET pulses, and then decrease during sunlight exposure, but based on a mass balance analysis on sorbed + aqueous TET concentrations, the data from the sorption extraction was considered unreliable (See Appendix One Section S1.4.3 for the mass-balance analysis).

Table 26: Summary of the best fit values obtained during manual fitting of first-order kinetic rates to the experimental pulse data.

Date pulse test started	HRAP[a]	k_{1L} 'Day 1' [b]	k_{1L} 'Day 2' [b]	K_d [b]	k_{1ds} [b]	R^2
		10^{-5} L µmol^{-1}	10^{-5} L µmol^{-1}	L g^{-1}	d^{-1}	
7-Aug-15	180 L	1.8	0.7	3.4	4.0	0.76
13-Aug-15	180 L	3	3.5	1.7	6.5	0.88
18-Aug-15	180 L	4		3.0	4.4	0.84
20-Aug-15	180 L	0.3	0.9	3.0	4.4	0.94
27-Aug-15	180 L	0.7	0.6	1.8	7.3	0.88
31-Aug-15	180 L		0.3	2.6	5.5	0.94
20-Mar-15	900 L		2.5	1.8	13.9	0.92[c]
25-Mar-15	900 L		2.7	3.0	8.3	0.72[c]

[a] Pulses of TET increased the TET concentration by 100 µg L^{-1} for the 180 L HRAP, and by 2 mg L^{-1} for the 900 L HRAP

[b] K_d is the partition coefficient, which was used to calculate the desorption kinetic rate k_{1ds}, k_{1L} is the 'photodegradation yield' during sunlight, fitted separately for TET removal in the light for 'Day 1' and 'Day 2'. The sorption kinetic constant k_{1s} was held constant at 15 d^{-1}.

[c] The reported R^2 values here for the 900 L HRAP only represent the fit to the TET removal during the second day. The initial TET concentrations in the first night immediately after the TET pulse was added were excluded, as these initial data points had very poor residuals.

K_d values are shown in Figure 28 to compare the partition coefficients calculated from batch tests, HRAP operation with continuous TET supply, and TET pulse tests in the pilot HRAPs. The range of fitted K_d partition coefficients listed in Table 26 based on the TET pulse tests were 1.7-3.4 L g^{-1}. This range of K_d values is similar to the continuous 4-day HRT HRAP operation with TET supply (K_d = 2.8 -4.6 L g^{-1}, Section 4.4.2) and is also similar to the lower range of the K_d values measured during the lab-scale HRAP operation (K_d = 1.0-12.2 L g^{-1} for all stages, with K_d = 1.0-3.3 L g^{-1} during Stages I to III; Section 4.1). The K_d for the 900 L pilot HRAP during continuous TET supply was K_d = 1.3 L g^{-1} (Section 4.4.1), which was lower than the K_d values fitted for the TET pulse tests in the pilot HRAPs, but was similar to the sorption batch tests (K_d = 1.4 ± 0.1 L g^{-1}; Section 4.3.2). Typical TET sorption K_d values were therefore in the 1.0-4.6 L g^{-1} range, if we exclude K_d values during Stages IV and V for the lab-scale

HRAP operation as outliers. K_d values reported for TET sorption to activated sludge are similarly variable: K_d = 0.47 L g^{-1} (Prado et al., 2009); K_d = 1.1 L g^{-1} (Plósz et al., 2010); K_d = 8.4-22.6 L g^{-1} (Kim et al., 2005).

k_{1L} constants are shown in Figure 28 to compare the 'photodegradation yields' calculated for the batch tests and the pulse tests.[a] The variation in k_{1L} values was greatest during the pulse tests in the 180 L pilot HRAP, with the fitted values for k_{1L} ranging from 0.3 $\times 10^{-5}$ to 4.0 $\times 10^{-5}$ L μmol^{-1}. The k_{1L} constants calculated for the 100 mL batch tests, 2.5 L batch tests, and the 900 L HRAP pulse tests were all within the range of variation in k_{1L} observed during the 180 L pilot HRAP pulse tests, except one 2.5 L batch test. As discussed in Section 4.3.1, the low k_{1L} values calculated for the autoclaved biomass in the batch tests (k_{1L} = 1.4×10^{-5}-1.7×10^{-5} L μmol^{-1}) compared with the active biomass (k_{1L} = 2.1×10^{-5}-6.0×10^{-5} L μmol^{-1}) may be due to increased light attenuation or due to lower pH or DO concentrations. The k_{1L} for the pulse tests in the 900 L HRAP (k_{1L} = 2.5×10^{-5}-2.7×10^{-5} L μmol^{-1}) were at the higher end of the range of k_{1L} for the 180 L HRAP pulse tests, which may be related to the high pH and DO concentrations during the 900 L HRAP pulse tests, based on the association between high pH and DO concentrations with higher TET removal rates discussed in Section 4.4.3.3.[b] Finally, the k_{1L} values calculated during the 100 mL batches (0.25×10^{-5} L μmol^{-1}) were most likely low due to the differences in light spectrum, as these tests were conducted using artificial light.

[a] There was too much uncertainty to calculate k_{1L} values during the HRAP operation with continuous TET supply.

[b] pH in the 900L HRAP peaked at 9.4 during the pulse tests, compared with peak pH up to 8 during the first two 180 L HRAP pulse tests, and peak pH ~7 for the last three pulse tests. Dissolved oxygen concentrations

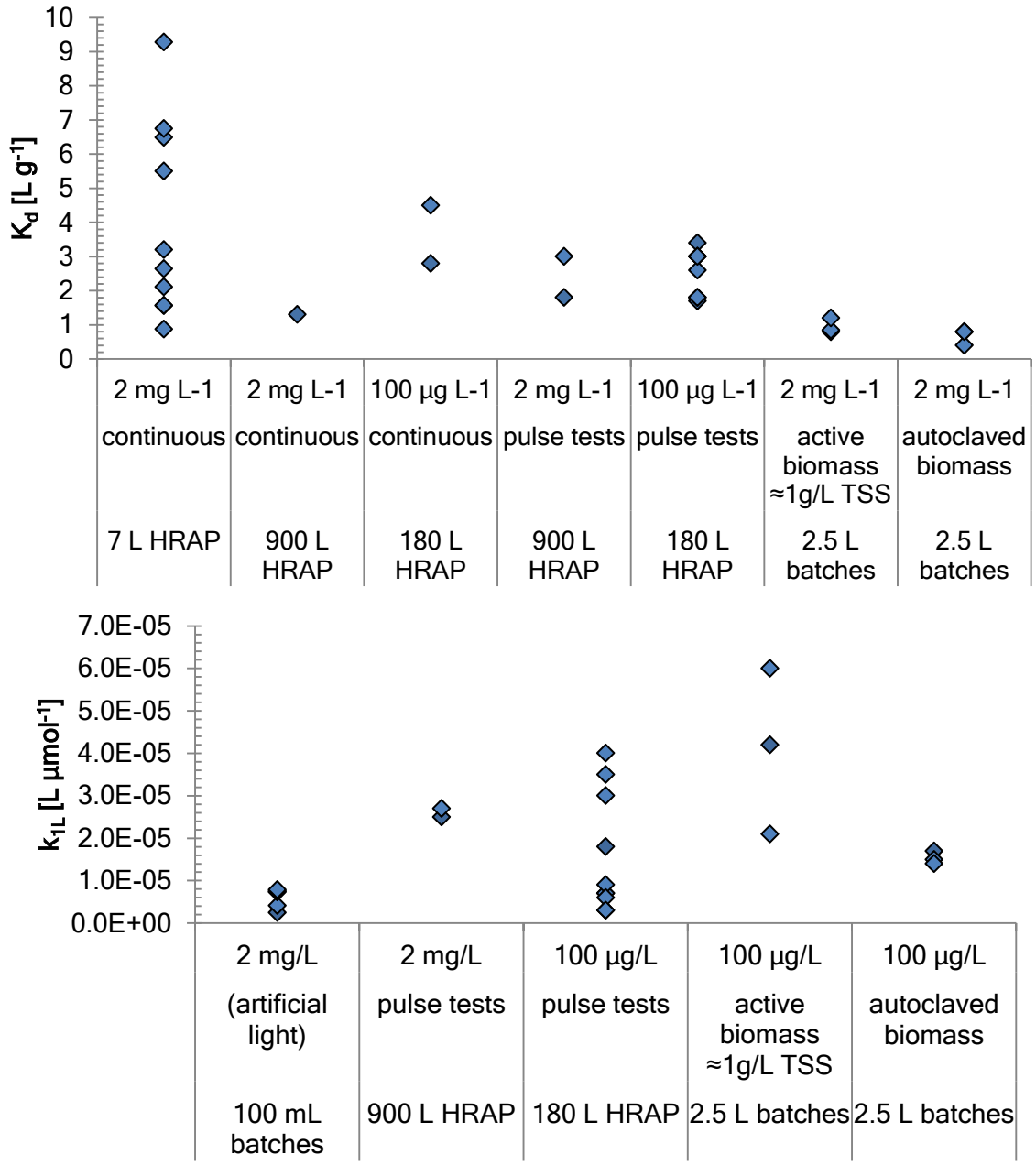

Figure 28: Dot-plot of the partition coefficient (K_d) and 'photodegradation yield' (k_{1L}) values calculated for the two batch methods and the pulse tests in the two HRAPs. The concentration values in the category labels indicate the initial TET concentrations for the experiments.

4.7 Batch experiments with antibiotic mixtures

Two short-term projects were conducted to study the fate of other antibiotics, in addition to the main study on TET. The results from these experiments are discussed in order to consider the influence of antibiotic structure on their fate in HRAPs.

4.7.1 Ciprofloxacin removal tests (work led by Andrea Hom-Diaz in NZ)

The first project was led by a PhD student, Andrea Hom-Diaz, Universitat de Autonoma de Barcelona, during a 6-month research period with our research team at Massey University.[a] Hom-Diaz focused on studying ciprofloxacin (CPX) removal, using similar experimental methods to those used for TET in NZ (7 L lab-bench HRAPs, 100 mL batch tests, and 900 L HRAP pulse tests). My contribution to this work was to advise Hom-Diaz in the planning of her experiments, setting up the experiment, and assisting with some analysis and data interpretation. The main conclusions from the work by Hom-Diaz are summarised in this section.

As in the case of TET removal, photodegradation was dominant for CPX removal in the light, and sorption was responsible for the removal under dark incubation (e.g. during batch tests with 0.3-0.4 g L^{-1} TSS, k_1 was 0.31 d^{-1} under 5.7 W m^{-2} light incubation, and 0.10 d^{-1} in the dark). The overall CPX removal rate (k_1 = 0.85 d^{-1} during sunlight, R^2 = 0.87) was lower than the TET removal rate (k_1 = 3.3-3.6 d^{-1} during sunlight, Section 4.4.3.1), during TET pulse tests in the 900 L HRAP. This lower CPX removal rate compared with TET was most likely due to the negative impact of biomass on CPX photodegradation (e.g. k_1 was 1.22 d^{-1} for HRAP filtrate in the light, compared with 0.31 d^{-1} at 0.3-0.4 g L^{-1} TSS).

[a] A journal article reporting this work is currently under review with Chemosphere; see Section 7

4.7.2 Antibiotic removal with 3-antibitotic mixtures

The second project investigating antibiotics other than just TET was entirely my own work, and was conducted during the 6 month research period in Spain. The experiments investigated two mixtures of three antibiotics each, using the outdoor batch test protocols (2.5 L batch reactors; Section 3.4.2.1). The mixtures considered were: 1) Sulfanilamide (SFL), TET, and Sulfamethoxazole (SMX); and 2) Sulfanilamide (SFL), Ciprofloxacin (CPX), and Chloramphenicol (CMP). These antibiotics were described in Section 3.5.1. Because the experimental methods were the same as the TET-only full-day batch tests, and the biomass was obtained from the same 180 L HRAP, a direct comparison was possible between the TET-only full-day batch experiments and the antibiotic mixture full-day batch experiments.

TET removal was similar to previous tests without the SFL and SMX (Figure 29). For TET removal in the SFL-TET-SMX test, the overall trends were similar to previous tests without the SFL and SMX (Figure 29), with sorption dominant during the night and photodegradation dominant during the daylight. The first-order kinetic TET removal rate in the light was k_1 = 11.0 d^{-1}, (k_{1L} = 0.86 ×10^{-5} L µmol^{-1}). This rate is similar to the TET removal during the "TET-only" batch test started on the 1st June 2015 (k_{1L} = 0.84 ×10^{-5} L µmol^{-1}), but the rate is much lower than the "TET-only" batch test started on the 5th Aug 2015 (k_{1L} = 1.4 ×10^{-5} L µmol^{-1}). This difference in TET removal could be due to interference from the presence of SFL and SMX, but is most likely due to normal variations observed between the batch tests. Photodegradation of SFL and SMX was observed in the MQ water control (SFL: 53% removal, SMX: 27% removal), but the concentrations of SFL and SMX in the presence of biomass were

highly variable[a], and thus no conclusions could be formed regarding the mechanisms of SFL and SMX removal in HRAPs from this batch test.

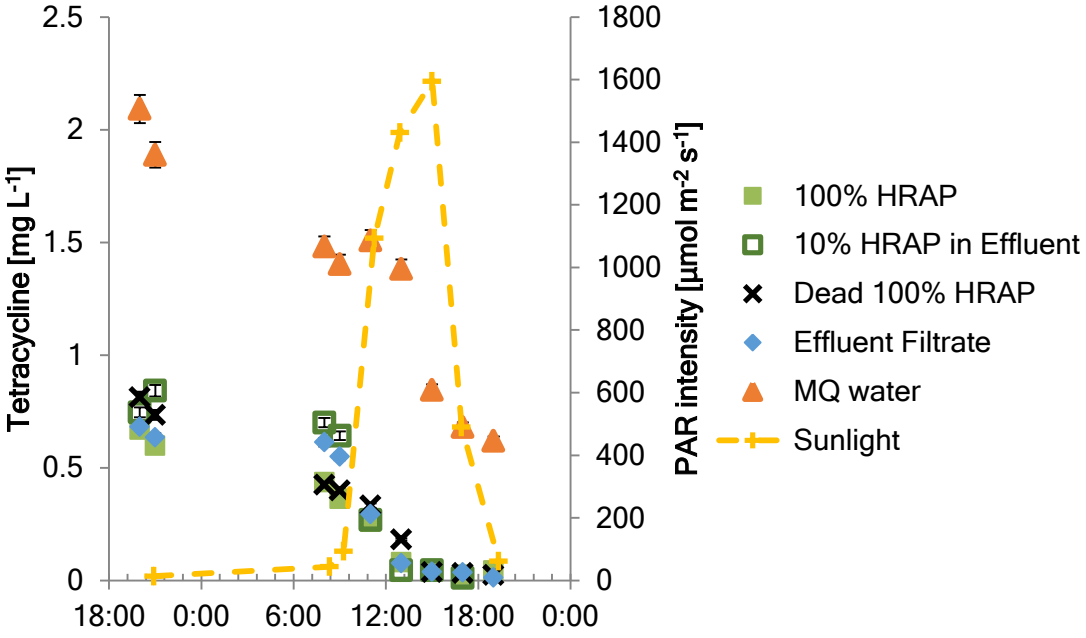

Figure 29: Removal of tetracycline in 2.5 L batch outdoors (start date 17th Aug 2015). Error bars show typical analysis error (see Table 15).

The results for the SFL-CPX-CMP mixture are shown in Figure 30, Figure 31, and Figure 32. The removal of SFL in the SFL-CPX-CMP test was less variable than the removal of SFL during the SFL-TET-SMX, perhaps due to preparation of the stock solution with 0.2% formic acid in order to dissolve the CPX.[b] Up to 50% aqueous SFL was removed (Figure 30), with evident photodegradation in MQ water. Rapid initial removal of SFL in the presence of 100% active HRAP biomass suggests that sorption was dominant. Therefore, direct photodegradation and sorption appear to be the main SFL removal mechanisms, and the presence of biomass prevented significant photodegradation.

[a] SMX concentrations in the presence of 100% active HRAP biomass were 0.05 – 0.5 mg L^{-1}; SFL concentrations in the presence of 100% active HRAP biomass were 0.35- 0.71 mg L^{-1}

[b] After CPX was dissolved, the pH of the stock solution was neutralized by addition of NaOH, before the stock solution was used in experiments, see Section 3.5.2

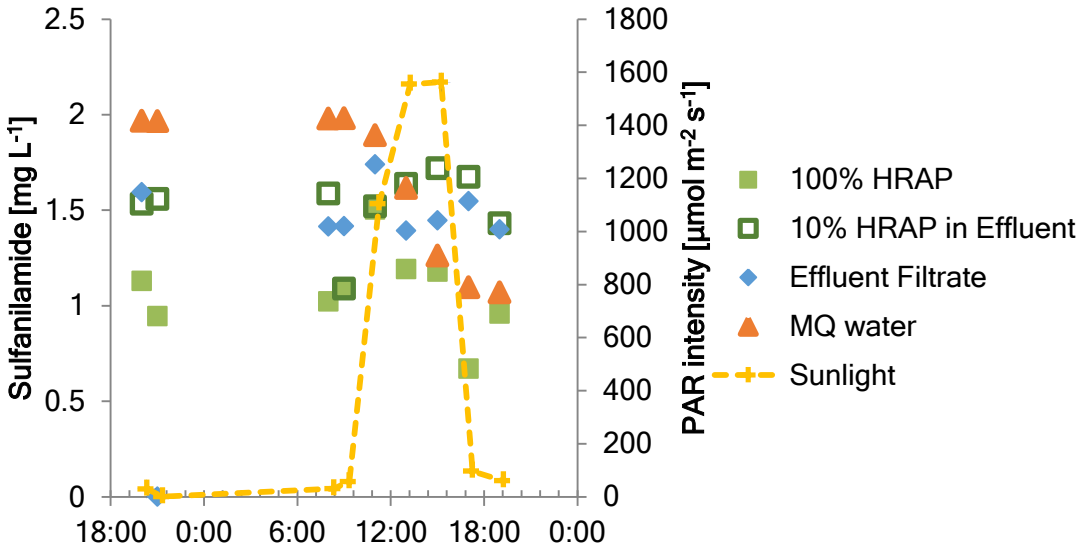

Figure 30: Removal of sulfanilamide in 2.5 L batch outdoors (start date 24th Aug 2015).[a]

The removal of CPX (Figure 31) was much higher than the removal of SFL, with essentially 100% removal for MQ water, and HRAP effluent filtrate. The presence of biomass appeared to reduce CPX removal during sunlight. CPX removal at night suggests that sorption is the dominant removal mechanism in the dark, with more sorption to active biomass than autoclaved biomass. The CPX removal rate was higher in the light for the autoclaved biomass than the active biomass (Table 27), which may be due to either differences in light attenuation, or higher CPX desorption from the active biomass. These results are similar to the results reported during 100 mL batch tests and the 900 L pilot HRAP in NZ (Section 4.7.1).

[a] The 'Dead biomass' condition is not shown for sulfanilamide, as another peak co-eluted with SFL during the HPLC analysis for samples from this condition, and thus prevented quantification. The chemical causing the interfering peak was apparently produced by the autoclaving of the biomass.

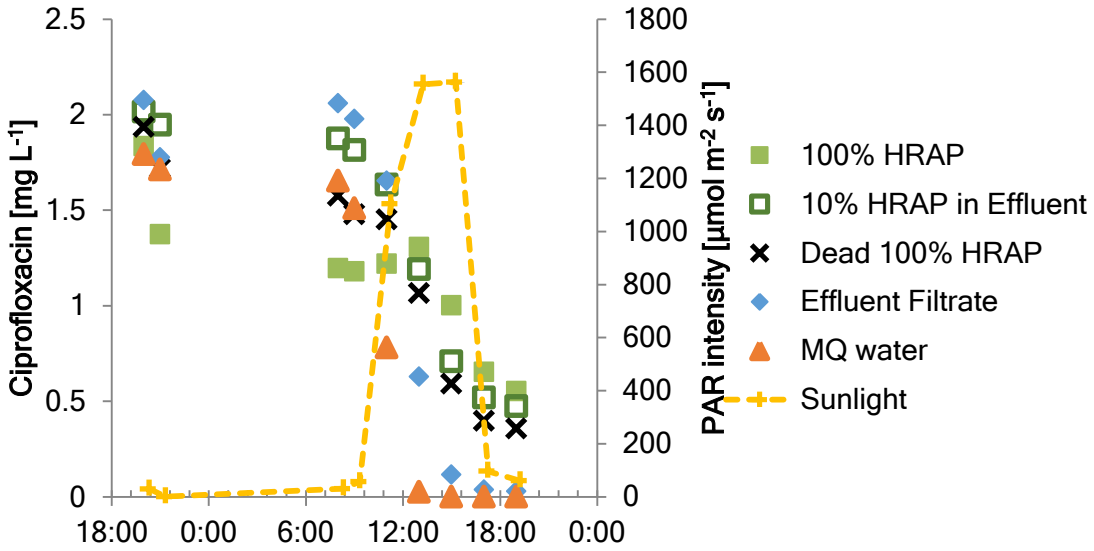

Figure 31: Removal of ciprofloxacin in 2.5 L batch outdoors (start date 24th Aug 2015).

In contrast to the removal of TET, SFL, and CPX, no significant sorption was observed during CMP removal at night (Figure 32). The removal of CMP in the light was slower overall than for CPX (Table 27), with 32% removal of CMP during sunlight in the presence of 100% active HRAP biomass. Similarly to the findings for CPX, the rate of CMP photodegradation was fastest in MQ water (90% removal). The presence of biomass reduced CMP removal, which indicates that direct photolysis was dominant. CMP removal was also reduced in the presence of effluent filtrate in comparison to the MQ water control, suggesting that CMP removal was more sensitive to light attenuation by dissolved organics.

Although our findings indicate that TET, CPX, and CMP may all be removed by photodegradation during WWT in HRAP, the photodegradation rates are significantly different, and the presence of biomass has a different effect on the removal of each antibiotic. Under these conditions, photodegradation rates in HRAP were, from the fastest to the slowest: TET>CPX>CMP>SFL (Table 27).

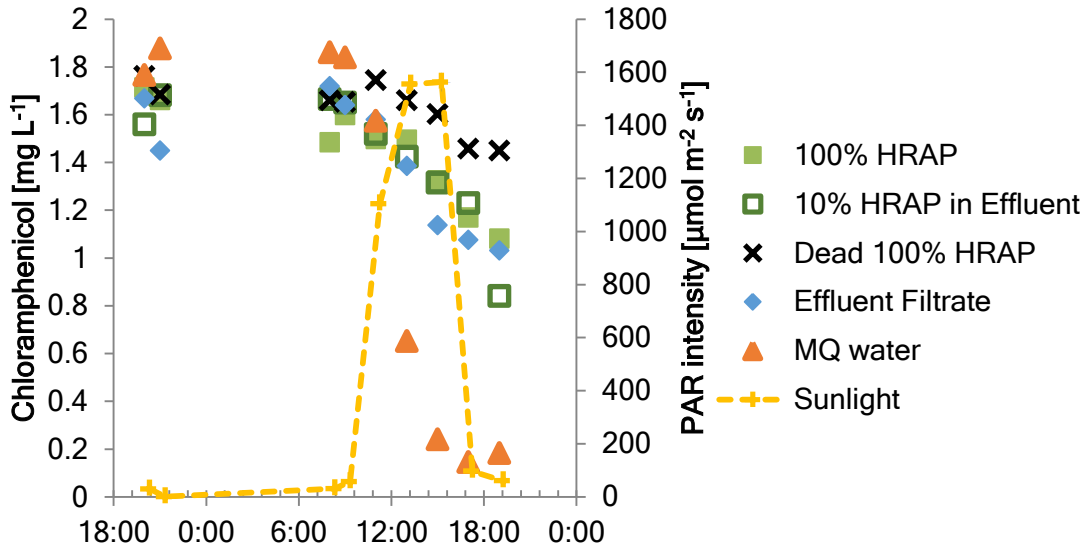

Figure 32: Removal of chloramphenicol in 2.5 L outdoor batches (start date 24th Aug 2015).

Table 27: Summary of pseudo-first order kinetic removal rates (11 am- 3pm) observed during the full-day outdoor batch test for the SCC mix of antibiotics. All values were calculated based on 3 data points.

	Tetracycline	**Sulfanilamide**	**Ciprofloxacin**	**Chloramphenicol**
	k_1 [d^{-1}] (R^2)	k_1 [d^{-1}] (R^2)	k_1 [d^{-1}] (R^2)	k_1 [d^{-1}] (R^2)
Active HRAP 100%	10.95 (0.98)	1.46 (0.07)	1.18 (0.96)	0.73 (0.77)
Active HRAP 10% in Effluent	10.60 (0.75)	-0.74 (0.88)	5.01 (0.92)	0.86 (1.00)
Active HRAP 10% in MQ	31.42 (1.00)	1.39 (0.49)	19.15 (0.82)	2.49 (0.92)
Dead HRAP 100%	13.04 (0.94)	0.98 (0.01)	5.37 (0.79)	0.51 (0.01)
Effluent Filtrate	12.13 (0.97)	1.11 (0.00)	15.97 (0.86)	1.97 (0.91)
MQ Water	2.14 (1.00)	2.43 (0.91)	41.13 (0.87)	11.29 (0.86)

5. Discussion of the overall implications for algal WWT ponds

Based on the results presented in this thesis, the removal of TET in HRAPs is dominated by photodegradation during daytime and by sorption to biomass during night time. Similar values of pseudo-first order photodegradation rates were recorded during operation of two pilot HRAPs in different countries, suggesting that the fate of TET in HRAPs is not sensitive to differences in biology or climate, provided key parameters such as sunlight and hydraulic design are taken into account.

The purpose of this section is to apply this knowledge to discuss the implications for antibiotic removal in full-scale algal WWT ponds. The discussion focuses on TET, using predictions from the kinetic model developed in Section 4.5.2 to estimate TET removal in the HRAP, facultative ponds, and maturation ponds.

The main limitations of this kinetic model are:

- The kinetic model was only partially validated: observed aqueous TET concentrations were within the range of predicted aqueous TET concentrations for the first three pulse tests, based on independent parameterization, but changing HRAP conditions resulted in observed TET removal lower than predicted values for the last three pulse tests in the 180 L HRAP (Section 4.5.2). In addition, sorbed TET concentrations were not validated. However, the model predicted the trends observed in the experimental data, accurately predicted the average aqueous TET concentration during 4 day HRT operation with continuous TET supply, and accounted for differences in TET removal caused by operation and design (Section 4.6). Therefore this model was still useful as a preliminary estimate of TET removal.

- The kinetic model did not include adjustment for pH, DO concentrations, or light attenuation. Based on results from batch experiments (Section 4.3) and TET pulse tests in the 180 L pilot HRAP (Section 4.4.3), it is likely that pH, DO concentrations, and light attenuation affected TET removal, but there was insufficient experimental data to improve the kinetic model accordingly.
- Facultative and maturation ponds have significant differences to HRAP, therefore a kinetic model developed for HRAP can only provide a rough prediction of removal in facultative and maturation ponds. Facultative ponds are biologically complex, often with anoxic and anaerobic microbial zones, and maturation ponds harbour a wide diversity of microbial species. Settling of dead biomass to the base of the ponds may also be important.
- The kinetic model does not account for biodegradation. Biodegradation of TET has been reported in some activated sludge systems (Cetecioglu et al., 2014; Chang et al., 2014; Song et al., 2016, 2015), and may therefore occur in HRAPs inoculated with a different algal-bacterial source, or in facultative and maturation ponds especially given the diverse ecology found in these systems.

The main assumptions used for the TET removal predictions were:

- The four systems can all be modelled as well-mixed systems, with no sludge recycle (SRT = HRT), and effluent flow equal to the influent flowrate ($Q_{in} = Q_{out}$).[a]
- Hydrolysis and biodegradation does not occur.
- All systems were operated outdoors, with the pond surface exposed to sunlight.

[a] Although the mixing in large ponds may be slow, the rate of mixing compared to the retention time of the system keeps ponds close to well-mixed.

The HRT, depth, and TSS concentrations used for the predictions are shown in Table 28, based on the range of values shown in Table 1.[a] The following constants were used for the simulation: $k_{1L} = 0.7 \times 10^{-5}$ L μmol^{-1} (conservative estimate, based on the lower range of k_{1L} values during 180 L HRAP pulse tests, Section 4.6); $k_{1s} = 15$ d^{-1} (from the sorption batch test, Section 4.3.2); $K_d = 2.8$ L g^{-1} (from the 4-day operation of the 180 L HRAP, Section 4.4.2); desorption kinetics were calculated based on K_d and TSS concentrations; $C_{in} = 100$ μg L^{-1} (the same influent concentration was used for the 180 L pilot HRAP, Section 4.4.2; however, since the kinetic model relies upon first-order kinetics the predicted TET removal efficiency percentages will not depend upon the influent concentration).

The data listed in Table 28 show that under these conditions, all the algal WWT ponds provide decent TET removal, with photodegradation contributing to the majority of TET removal. TET sorption contributed ~7% for all three algal WWT ponds – the contribution of sorption would increase if TSS concentrations were higher, or if TET removal by photodegradation was reduced. The greater depth of facultative ponds and maturation ponds compared with the HRAP reduces TET removal, but the longer HRT for facultative ponds partially compensates for the reduction of surface area for photodegradation. Although the predicted TET removal for the maturation ponds is lower than for the other two algal WWT ponds, maturation ponds typically receive WW effluent from facultative ponds or HRAPs. In a waste stabilisation pond system with a facultative and maturation ponds arranged in a series, therefore, the total TET removal could be much higher: The facultative pond would remove the majority of the TET in the influent, and the maturation pond would further lower the TET effluent

[a] Table 1 in Section 2.2 reports typical design and operational parameters for HRAP, facultative ponds, and maturation ponds, based on referenced literature.

concentration for total TET removal of 84% based on the numbers in Table 28.[a] If the removal of antibiotics by photodegradation is reduced in the presence of high biomass concentrations (e.g. ciprofloxacin and chloramphenicol, Section 4.7), the removal of these antibiotics may be greater in maturation ponds than in facultative ponds or HRAP.

Table 28: Predicted TET removal in three algal WWT ponds,[a] based on the kinetic model developed in this thesis.

	Pond Type	HRAP	Facultative Pond	Maturation Pond
Design and operation set-points	HRT [d]	7	30	7
	Biomass conc. [g L^{-1}]	0.2	0.1	0.05
	Depth [m]	0.25	1.5	1.2
	Area [m^2][b]	2800	2000	583
Predicted TET aqueous and sorbed concentrations based on the above set-points:				
TET aqueous conc. [µg L^{-1}]	Mean	11	22	48
	Max	14	23	50
	Min	9	22	47
TET sorbed conc. [mg g^{-1}]	Mean	32	63	135
	Max	38	64	140
	Min	26	61	131
Percentage aqueous TET removed	Mean	89%	78%	52%
Percentage TET sorbed	Mean	6.3%	6.3%	6.8%
Percentage TET photodegraded	Mean	82%	71%	45%

[a] Parameters were taken from mid-range values in Table 1 (references in Table 1). Daily sunlight intensity was based on data recorded on 14th Aug 2014, which had the lowest average sunlight intensity recorded during the pulse tests.
[b] Area is based on a constant WW influent of 100 m^3 d^{-1}. Predicted TET concentrations do not change when influent flow-rate is changed, if area and volume are re-calculated based on the depth and HRT.

[a] Calculating total TET photodegradation in a serial facultative pond system, based on one facultative pond and one maturation pond in series, there should be a total TET removal of 71% (facultative) × 45 % (maturation) = 84% total removal. TET removal by sorption was not included in this calculation, as TET desorption from the biomass could return TET to the wastewater.

In comparison, the overall TET removal typically observed in activated sludge WWT varies broadly (e.g. 32-85% (Batt et al., 2007); 10-85% (Plosz et al., 2010); 24-100% (Michael et al., 2013)). Since TET sorption is generally considered the main removal mechanism in activated sludge (Kim et al., 2005; Li and Zhang, 2010), net sludge productivity should be the key factor in TET removal by activated sludge, with greater TET removal when more solids are removed from the system rather than re-digested. Variation in TET removal may also occur due to differences in water hardness, both in activated sludge WWT and algal WWT ponds, as TET may precipitate with Ca^{2+}, Mg^{2+} or other cations (Daughton and Ternes, 1999).

Because the outdoor experimental work presented in thesis was predominantly conducted under direct sunlight during summer, caution must be used in applying results to the estimation of TET fate in the winter season and cloudy conditions. A sensitivity analysis for the 180 L HRAP was performed (Appendix Five Section S5.4), and concluded that TET removal in the HRAP was most sensitive to changes in lighting conditions and photodegradation constants. The key next steps in improving the estimation of TET fate in algal WWT pond systems are therefore to identify the main causes for the differences in photodegradation rates reported (Section 4.5) especially considering light attenuation (e.g. by changes in TSS concentrations) and pH changes, and to investigate how the photodegradation rates change under different light conditions such as cloudy weather.

Compared to other WWT systems algal WWT ponds should remove TET efficiently due to the photodegradation of TET. The removal of TET in algal ponds is highest for those ponds with high surface-area-to-volume ratios; therefore TET removal should be highest in shallow ponds. However, because TET removal is highly dependent upon photodegradation in algal WWT, seasonal variation in TET removal efficiency is likely, with high TET removal in summer and lower TET removal in winter.

6. Conclusions

In this thesis, high TET removal (85 to >98%) was demonstrated in lab-scale and pilot-scale HRAPs under a variety of operating conditions (4, 7 and 9 days hydraulic retention time (HRT)), in both Oceanic (NZ) and Mediterranean (Spain) climates, at 2 mg L^{-1} and 100 µg L^{-1} influent TET concentrations in the WW, and with different dominant algae species in the HRAPs. The similar TET removal efficiencies observed across the range of conditions tested increases the reliability of the results for estimating TET removal in HRAPs with different operation and design.

The TET removal mechanisms (photodegradation, sorption, biodegradation, and hydrolysis) were successfully investigated under conditions relevant to HRAPs under both artificial light and sunlight. During investigation of these mechanisms, results also suggested that TET removal was affected by pH changes and TSS concentrations in the HRAPs.

Photodegradation was the most quantitatively significant mechanism of TET removal in HRAPs, both during batch tests and during continuous pilot HRAP operation. During batch tests conducted under artificial light or sunlight, pseudo-first order rates were commonly 10-20 times greater during light exposure than during dark conditions. Due to the dominant impact of photodegradation, a statistically significant ($p < 0.01$) difference was observed between the TET concentrations in the morning (4.2 ± 0.5 µg L^{-1}) and evening (2.5 ± 0.2 µg L^{-1}) during monitoring of the 180 L pilot HRAP with continuous 100 µg L^{-1} TET supplied in the WW influent.

Quantum yields for TET photodegradation had a pseudo-first-order dependence upon aqueous TET concentrations in both batch experiments and TET pulse tests in pilot HRAP. During these pulse tests, photodegradation kinetic constants calculated for TET

removal rates in the light at pH greater than 7 were typically double the TET removal rates at pH < 7. This increase in TET removal at higher pH is reasonable based on the literature, as TET has a pKa at ~7.8, and thus the ionic form of TET in solution would start to change at pH ~7, and increased TET photolysis has been reported at pH > 7. However, high pH was also correlated with high DO concentrations, high light exposure, and high temperature. Changes in light attenuation or WW characteristics may also have contributed to the decrease in TET photodegradation rates. Results from batch tests with manual adjustment of pH showed that high pH caused a rapid decrease (<1 min) in TET concentrations that could not be related to a particular mechanism. Further investigation into the effect of pH upon TET removal is required.

Indirect photodegradation appeared to be dominant over direct photolysis for the photodegradation of TET. A decrease is TET removal was associated in some cases with high concentrations (~ 1 g L^{-1} TSS) of biomass, but TET photodegradation in the presence of biomass or effluent was 3-7 times higher than photodegradation of TET in MQ water, where only direct photolysis could occur.

Sorption was a low contributor to overall TET removal (e.g. 3-10% removal by sorption to biomass in lab and pilot scale HRAPs). During batch tests, a partition coefficient (K_d, [mg g^{-1} sorbed TET]/[mg L^{-1} aqueous TET]) was estimated to be 1.4 ± 0.1 L g^{-1} (std. error of regression, n=3). However, the sorption partition coefficients determined during continuous operation were K_d = 1.3 L g^{-1} (900 L HRAP), and K_d = 2.8 or 4.6 L g^{-1} (180 L HRAP). Sorption was especially influential during the pulse tests, with ~50% removal occurring by TET sorption. In the absence of photodegradation, it is likely that sorption would dominate the fate of TET in HRAPs as is the case in conventional systems such as activated sludge. If sludge with sorbed antibiotics is digested, the antibiotics may desorb and return to WW, and if sludge with sorbed antibiotics is spread on farmland,

the presence of sorbed antibiotics may encourage antibiotic resistance. Therefore, the lower overall contribution of sorption due to the photodegradation in HRAPs means that risks regarding disposal or usage of algal biomass are lower than risks from TET in waste activated sludge.

Biodegradation was not shown to be significant, but cannot be fully excluded. Under batch test conditions, the influence of biodegradation appeared negligible. Any differences between autoclaved and active biomass conditions during batch tests differences were best explained by changes to the sorption properties of the biomass due to autoclaving. The autoclaving of the biomass also reduced TET removal under sunlight, most likely due to differences in light penetration between the autoclaved and active biomass conditions.

Nevertheless, TET removal was reduced during disruptions to the algal-bacterial biomass by rotifers (biomass grazers). The reduction in TET removal associated with the presence of rotifers could be due to reduced algal or bacterial activity which 1) reduced the removal of organics in the WW thus affecting light attenuation, and 2) changed the diurnal variations in pH and DO concentrations.

No obvious changes were noted during addition of TET to the WW influent, but during the period of operation where pulses of TET were added to the 180 L HRAP, the settleability of the HRAP biomass decreased, and the biomass formed more biofilms in the HRAP.

Irreversible hydrolysis was not observed during this thesis. The TET did form the epimer 4-epi-TET in solution, but this was considered equivalent to the parent TET in all calculations, since it was a reversible relationship.

Preliminary investigations into the antibiotic removal mechanisms for sulfanilamide, ciprofloxacin, and chloramphenicol in the presence of algal-bacterial biomass found that sulfanilamide and ciprofloxacin were removed by sorption at night. No sorption was observed for chloramphenicol during the night. These three antibiotics were also removed by photodegradation in MQ water, but the presence of biomass reduced their photodegradation, especially for chloramphenicol and sulfanilamide. In the presence of algal-bacterial biomass (TSS ≈ 1.1 g L^{-1}), photodegradation rates were, from fastest to slowest: TET>CPX>CMP>SFL.

This research has provided a range of pseudo-first-order kinetics for the dominant TET removal mechanisms, and this provides a foundation for future research on TET fate in algal WWT ponds. A kinetic model was developed and used to predict TET removal in the pilot HRAPs using parameters derived from batch experiments. The model predictions for aqueous TET concentrations were successfully validated against initial TET pulse tests in the 180 L pilot-scale HRAP. However TET removal decreased in subsequent pulse tests in the pilot HRAP, resulting in over-prediction of TET removal by the kinetic model. This decrease in TET removal was associated with decrease in pH, DO concentrations, and biomass settleability, but causal relationships between TET removal and these variables could not be quantified. Batch test results supported the idea that pH and light attenuation had an effect on TET removal, but there was insufficient data to predict effects of pH and light attenuation.

To further develop the predictive model for TET removal in algal WWT ponds:

- TET desorption during photodegradation under sunlight should be confirmed and desorption kinetics should be quantified using experimental methods such as stirred flow cells.

- Further research is required to investigate if changes in pH, DO concentrations, and light attenuation can indeed explain the variation observed in the TET removal during the pulse tests. Accounting for pH, DO concentrations, and light attenuation should improve the robustness of the model to predict TET removal across different systems.
- To improve TET removal predictions throughout all seasons (winter as well as summer) and different climates, TET removal should be investigated under a greater range of light intensities.
- Given the wide usage of facultative and maturation ponds, TET removal should be investigated in these algal WWT systems.

Until further development of the model can occur, the predictive model is still useful as a preliminary estimate of TET removal in algal WWT ponds.

The combination of batch and continuous trials presented in this thesis provides a step forward in the understanding of TET fate in algal WWT ponds reported by de Godos et al. (2012), and complements the wider area of emerging contaminant research such as the batch and continuous studies of other pharmaceuticals in HRAPs (Matamoros et al., 2016, 2015b). It is hoped that this research will be further used to develop the understanding of TET fate in other algal WWT ponds also, and complemented by similar studies on other antibiotics. Further research is also needed to understand how antibiotic resistance in HRAPs develops/changes with different antibiotic concentrations in the influent WW, as antibiotic resistance is the major concern related to the release of antibiotics into the environment.

7. Record of publications and presentations

Publication type	Title	Status	Publisher/Organization
Journal Article: Literature review	Emerging contaminant degradation and removal in algal wastewater treatment ponds: identifying the research gaps	Published[a]	Journal of Hazardous Materials
Journal Article: Research article	Photodegradation and sorption govern the fate of tetracycline in high rate algal ponds fed with real wastewater	Drafting for submission	Bioresource Technology
Conference presentation #1 (Hamilton 2014)	Removal of an antibiotic in algal wastewater treatment systems: tetracycline	Aug '14, completed	Water NZ
Conference Presentation #2 (Sweden 2015)	Removal of tetracycline in continuous HRAPs	Conference date 7-10th Jun	IWA
Conference Poster (Barcelona)	Removal of tetracycline in continuous HRAPs	Conference Date 3rd-7th May	Society of Environmental Toxicology and Chemistry (SETAC)
Book chapter (my contribution was to write a page describing emerging pollutant fate in algal WWT, and assisting with editing)	Chapter 3 - Microalgae cultivation in wastewater. In: Microalgae-based biofuels and bioproducts	Submitted for publication	Elsevier
Unrelated Extra Paper #1	*A critical assessment of the feasibility of sequential chemical-biological processes for the treatment of inhibitory wastewater effluents.*	*Published 2014*[b]	*Journal of Hazardous Materials*
Unrelated Extra Paper #2	*Nitrous oxide emissions from high rate algal ponds treating domestic wastewater*	*Published 2015*[c]	*Bioresource Technology*
Paper with Hom-Diaz, A. (collaborative work)	Ciprofloxacin removal during secondary domestic wastewater treatment in high rate algal ponds	*Under review by journal*	*Chemosphere*

[a] Norvill, Z.N., Shilton, A., Guieysse, B., 2016. Emerging contaminant degradation and removal in algal wastewater treatment ponds: Identifying the research gaps. J. Hazard. Mater. 313, 291–309. doi:http://dx.doi.org/10.1016/j.jhazmat.2016.03.085

[b] Guieysse, B., Norvill, Z.N., 2014. Sequential chemical-biological processes for the treatment of industrial wastewaters: Review of recent progresses and critical assessment. J. Hazard. Mater.

[c] Alcántara, C., Muñoz, R., Norvill, Z., Plouviez, M., Guieysse, B., 2015. Nitrous oxide emissions from high rate algal ponds treating domestic wastewater. Bioresour. Technol. 177, 110–117. doi:10.1016/j.biortech.2014.10.134

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Appendices for:

Characterizing the Removal of Antibiotics in Algal Wastewater Treatment Ponds: A Case Study on Tetracycline in HRAPs

A thesis presented in partial fulfilment of the requirements for the degree of

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in

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Table of Contents

S1. Appendix One – Pond Performance data

S1.1 Lab scale HRAPs

S1.1.1 Algal identification

Overall comments: Diversity decreased slightly as the HRT was decreased. Of the dominant species (*Pediastrum, Scenedesmus*, *Microcystis*), HRAP B remained steady with *Scenedesmus* and *Microcystis*, but dropped in *Pediastrum*. In contrast, HRAP A dropped in *Scenedesmus* .and *Microcystis*, but increased in *Pediastrum*. *Pseudosphaerocystis* was the only one to appear in the list for 4 day HRT and not 7 day HRT. *Gomphonema* was the only other one to increase (HRAP B only) when shifting to 4 day HRT.

Table 1: Relative abundance of phototroghs (algae and cyanobacteria) in the lab-scale HRAPs during different periods of operation. Analysis performed by Cawthron. Ratings for relative abundance: 1 Rare, 2 Rare-Occasional, 3 Occasional, 4 Occasional-Common, 5 Common, 6 Common-Abundant, 7 Abundant, 8 Dominant.

Relative abundance	**Date**	**25-Aug-14**	**25-Aug-14**	**15-Sep-14**	**15-Sep-14**	**5-Nov-14**	**5-Nov-14**	**17-Dec-14**	**17-Dec-14**
Species	Type	HRAP-A 7 day HRT	HRAP-B 7 day HRT	HRAP-A 4 day HRT	HRAP-B 4 day HRT	HRAP-A 4 Day HRT 8 Hours light	HRAP-B 4 Day HRT 8 Hours light	HRAP-A 4 Day HRT 16 Hours light	HRAP-B 4 Day HRT 16 Hours light
***Chlamydomonas* sp.**	Chlorophyta								2
***Cryptomonas* sp.**	Chlorophyta					2	1		
***Golenkinia* sp.**	Chlorophyta	1							
***Monoraphidium* sp.**	Chlorophyta	2	4						
***Monoraphidium* spp.**	Chlorophyta						4		
***Oedogonium* sp.**	Chlorophyta						2	3	6
***Pediastrum* sp.**	Chlorophyta	7	5	8	3	7	3		2
***Pseudosphaerocystis* sp.**	Chlorophyta			1	5				
***Romeria* sp.**	Chlorophyta							1	
***Scenedesmus* sp.**	Chlorophyta	5	7	2	7	5	2	8	8
***Stigeoclonium* sp.**	Chlorophyta								4
Unknown colonial *Chlorophyte*	Chlorophyta					8	6	7	7
Pseudanabaenaceae	Cyanobacteria (not known to be toxic)	2	3		2				
***Aphanocapsa* sp (<1um)**	Cyanobacteria (potentially toxic)						8		
***Microcystis* spp.**	Cyanobacteria (potentially toxic)	8	8	5	8				
Oscillatoriales Unid.	Cyanobacteria (potentially toxic)	2							

***Phomidium* sp.**	Cyanobacteria (potentially toxic)		1						
***Pseudanabaena* sp.**	Cyanobacteria (potentially toxic)	6	6	3		2	2	2	1
***Fragilaria* sp.**	Diamtoms	4	2		1				
***Gomphonema* sp.**	Diamtoms	2	2	2	6	1	6		3
***Navicula* sp.**	Diatoms						1		
***Nitzschia* sp.**	Diatoms	3	1						
Reimeria sinuata	Diatoms							1	
***Synedra* sp.**	Diatoms							1	
***Peridinium* sp.**	Dinoflagellates							1	
***Peridinium* sp.**	Dinoflagellates						1		
Small unicells	Non-Toxic species	4	5		4	6	5	5	4
	Total number of species listed	12	11	6	8	7	12	9	9

S1.1.2 General monitoring parameters

Table 2: Summary of the environmental conditions and effluent composition under the 6 different operating stages of the lab-scale HRAP, additional to the data presented in the main thesis text. Errors are given as 95% confidence intervals of the sample data. Some general monitoring parameters are given as a range since data was not universally stable. [TOC=Total Organic Carbon, DO=Dissolved Oxygen]

Period	**Period I, 9th Jul to 28th Jul**		**Period II, 4th Aug to 27th Aug**		**Period III, 1st Sep to 15th Sep**		**Period IV, 10th Oct to 22nd Oct**		**Period V, 24th Oct to 29th Oct**		**Period VI, 10th Nov to 29th Nov**		**Period VII, 5th Dec to 18th Dec**	
Pond Name	HRAP A	HRAP B	HRAP A	HRAP B	HRAP A	HRAP B	HRAP A	HRAP B	HRAP A	HRAP B	HRAP A	HRAP B	HRAP A	HRAP B
HRT	7	7	7	7	4	4	7	7	7	7	4	4	4	4
Light Hours/ 24 h	12	12	12	12	12	12	8	8	8	8	8	8	16	16
Effluent	Overflow	Overflow	Batch Siphon	Batch Siphon	Batch Siphon	Batch Siphon	Batch Siphon	Batch Siphon	Batch Siphon	Batch Siphon	Batch Siphon	Batch Siphon	Batch Siphon	Batch Siphon
Influent	WW (no Tet)	Mixed Tet/WW	Mixed Tet/WW	Mixed Tet/WW	Mixed Tet/WW	Mixed Tet/WW	Mixed Tet/WW	Mixed Tet/WW	Split Tet/WW	Split Tet/WW	Split Tet/WW	Split Tet/WW	Split Tet/WW	Split Tet/WW
T AM [°C]	(0)	(0)	16.0 to 17.3 (14)	15.6 to 16.9 (14)	16.2 to 17.7 (11)	15.8 to 17.3 (11)	14.7 to 18.2 (17)	16.4 to 17.7 (9)	16.4 to 17.6 (4)	16.0 to 17.3 (4)	16.6 to 18.9 (8)	16.2 to 18.8 (8)	17.2 to 19.2 (11)	17.0 to 19.0 (11)
T PM [°C]	22.9 to 24.8 (14)	22.9 to 24.7 (14)	22.1 to 24.1 (16)	22.2 to 24.0 (16)	23.0 to 23.7 (10)	23.0 to 23.8 (10)	21.9 to 24.0 (15)	22.2 to 24.2 (8)	22.8 to 23.7 (3)	22.9 to 24.0 (3)	21.7 to 24.2 (15)	21.7 to 24.7 (15)	21.6 to 24.5 (9)	22.1 to 24.6 (9)
TOC Effluent [mgC/L]	195 ± 59 (9)	180 ± 71 (9)	173 ± 38 (11)	128 ± 21 (11)	128 ± 42 (7)	84 ± 24 (7)	146 ± 22 (11)	108 ± 22 (6)	79 ± 12 (3)	83 ± 71 (3)	49 ± 8 (9)	49 ± 8 (9)	111 ± 37 (6)	81 ± 23 (6)
TOC Filtered Effluent [mgC/L]	24 ± 3 (9)	23 ± 3 (9)	27 ± 4 (11)	27 ± 5 (11)	31 ± 5 (7)	29 ± 3 (7)	19 ± 5 (11)	19 ± 2 (5)	19 ± 3 (3)	19 ± 3 (3)	15 ± 2 (9)	16 ± 1 (9)	17 ± 2 (6)	15 ± 1 (6)

Period	Period I, 9th Jul to 28th Jul		Period II, 4th Aug to 27th Aug		Period III, 1st Sep to 15th Sep		Period IV, 10th Oct to 22nd Oct		Period V, 24th Oct to 29th Oct		Period VI, 10th Nov to 29th Nov		Period VII, 5th Dec to 18th Dec	
TN Effluent [mgN/L]	43 ± 8 (9)	42 ± 10 (9)	36 ± 6 (11)	27 ± 3 (11)	32 ± 6 (7)	31 ± 4 (7)	35 ± 3 (11)	39 ± 4 (6)	30 ± 12 (3)	36 ± 8 (3)	37 ± 2 (9)	40 ± 4 (9)	37 ± 11 (6)	28 ± 14 (6)
TN Filtered Effluent [mgN/L]	15 ± 3 (9)	17 ± 4 (9)	12 ± 3 (11)	10 ± 2 (11)	17 ± 1 (7)	23 ± 2 (7)	19 ± 1 (10)	24 ± 4 (5)	27 ± 1 (3)	33 ± 2 (3)	29 ± 3 (9)	33 ± 4 (9)	20 ± 5 (6)	22 ± 7 (6)
$N\text{-}NO_3^-$ Filtered Effluent [mg/L]	2.6 to 11.1 (9)	2.4 to 11.5 (8)	2.7 to 14.9 (11)	1.1 to 7.4 (12)	5.2 to 9.4 (7)	2.8 to 10.6 (7)	10.9 to 18.1 (10)	18.5 to 24.9 (10)	22.2 to 27.4 (3)	30.1 to 32.6 (3)	22.3 to 26.7 (9)	25.7 to 30.7 (9)	11.0 to 20.6 (6)	12.7 to 22.0 (6)
$N\text{-}NO_2^-$ Filtered Effluent [mg/L]	2.28 to 9.75 (9)	2.01 to 9.79 (9)	1.73 to 3.34 (12)	3.35 to 8.28 (12)	5.05 to 9.93 (7)	10.76 to 17.59 (7)	2.13 to 3.14 (10)	1.15 to 2.51 (10)	0.81 to 1.48 (3)	0.59 to 0.75 (3)	0.08 to 0.65 (9)	0.25 to 0.65 (9)	0.30 to 1.17 (6)	0.37 to 0.60 (6)
$P\text{-}PO_4^-$ Filtered Effluent [mg/L]	0.31 to 1.23 (8)	0.14 to 0.71 (8)	0.00 to 0.45 (12)	0.00 to 0.23 (12)	0.00 to 0.24 (7)	0.16 to 0.96 (7)	0.00 to 1.10 (10)	1.00 to 1.59 (10)	1.14 to 1.69 (3)	1.44 to 1.81 (3)	0.92 to 2.28 (9)	1.11 to 1.67 (9)	0.18 to 0.93 (6)	0.19 to 0.63 (6)
%Photo-synthetic Efficiency	1.9 ± 0.4%	1.8 ± 0.2%	3.4 ± 0.6%	2.9 ± 0.6%	2.8 ± 1.3%	1.7 ± 0.5%	3.2 ± 0.7%	2.9 ± 0.5%	1.9 ± 1.4%	2.1 ± 1.3%	0.4 ± 0.1%	1.0 ± 0.1%	2.4 ± 0.8%	2.7 ± 1.2%

S1.1.3 Sorption Data

Table 2: Sorption extraction results on biomass taken from lab-HRAP effluent. There is more uncertainty with the results from the 1st and 25th of Aug, as later tests showed that longer extraction times (>10 hours) were necessary for the biomass-sorbed-tetracycline to reach equilibrium with the extraction solvent.

Test [Pond, Date]	HRT [d]	Light [h/d]	Biomass conc. TSS [g/L]	Hours extracted	$mg_{tetracycline}/mg_{biomass}$	Kd [L/g]	Contribution to removing the 2 mg/L tetracycline in influent	Average recovery efficiency of the spiked standards
HRAP A 1st Aug	7	12	0.90	2	0.11	0.78	3.4%	78%
HRAP B 1st Aug	7	12	0.74	2	0.18	1.73	4.6%	89%
HRAP A 25th Aug	7	12	0.48	2	0.22	1.56	3.6%	81%
HRAP B 25th Aug	7	12	0.31	2	0.31	3.20	3.5%	79%
HRAP A 1st Sep	4	12	0.31	10	0.50	2.11	6.9%	77%
HRAP B 1st Sep	4	12	0.23	10	0.56	2.52	5.9%	67%
HRAP B 8th Sep	4	12	0.14	14	0.62	2.77	4.1%	90%
HRAP A 2nd Oct	7	8	0.39	22	0.24	1.31	4.6%	74%
HRAP B 2nd Oct	7	8	0.36	22	0.21	0.87	3.7%	80%
HRAP A 22nd Oct	7	8	0.22	21	0.30	1.57	3.2%	72%
HRAP B 22nd Oct	7	8	0.36	21	0.39	0.87	7.0%	68%
HRAP A 20th Nov AM	4	8	0.11	21	1.73	6.49	9.4%	92%
HRAP A 20th Nov PM	4	8	0.17	21	1.40	6.83	9.8%	91%
HRAP B 20th Nov AM	4	8	0.14	21	1.82	5.50	15.4%	91%
HRAP B 20th Nov PM	4	8	0.13	21	1.80	7.12	11.5%	91%
HRAP A 15th Dec AM	4	16	0.18	21	0.77	9.28	9.0%	90%
HRAP A 15th Dec PM	4	16	0.19	21	0.95	7.53	5.6%	95%
HRAP B 15th Dec AM	4	16	0.14	21	0.94	6.74	6.8%	91%
HRAP B 15th Dec PM	4	16	0.13	21	0.88	7.21	6.6%	95%

S1.2 900 L HRAP

S1.2.1 Algal identification

Table 3: Relative abundance of phototroghs (algae and cyanobacteria) in the 900 L pilot-scale HRAP on 3 different dates. Analysis performed by Cawthron. Ratings for relative abundance: 1 Rare, 2 Rare-Occasional, 3 Occasional, 4 Occasional-Common, 5 Common, 6 Common-Abundant, 7 Abundant, 8 Dominant.

	17-Dec-14	**9-Mar-15**	**27-Jan-15**
***Actinastrum* sp.**		2	1
***Chlamydomonas* sp.**	4		
***Closterium* sp.**	1		1
***Coelastrum* sp.**	4		3
***Crucigeniella* sp.**	1		
***Cryptomonas* sp.**	1		
***Dictyosphaerium*, sp.**	1	7	2
***Kirchneriella* sp.**	1		
***Micractinium* sp.**			1
***Monoraphidium* sp.**	4	1	2
***Oocystis* sp.**	6	4	6
***Pediastrum* sp.**	2	8	8
***Pteromonas* sp.**	3		
***Scenedesmus* sp.**	8	7	4
***Stigeoclonium* sp.**		1	
Unknown colonial Chlorophyte	1		2
Pseudanabaenaceae	2		
***Aphanocapsa* sp (<1um)**	3		2
***Microcystis* spp.**			1
***Planktothrix* sp.**		6	5
***Pseudanabaena* sp.**	7		1
***Gomphonema* sp.**		4	3
***Navicula* sp.**			
***Nitzschia* sp.**		2	5
***Synedra* sp.**			2
***Peridinium* sp.**	1	3	
Small flagellates (<5um)	3		
Small unicells	5	5	7
Total number of species listed	19	11	17

S1.2.2 General monitoring parameters

Table 4: Average HRAP characteristics for the 900 L HRAP at PNCC, Palmerston North, NZ, Feb-April 2015

Parameters	Average	Standard Deviation	Count
COD[mg/L]	506	103	4
COD filtered [mg/L]	78	18	3
TSS [mg/L]	421	88	30
N-Nitrite [mg/L]	11.6	4.6	9
N-Nitrate [mg/L]	0.4	0.2	15
P-Phosphate [mg/L]	1.5	0.4	12
TOC [mg/L]	128	27	18
TOC filtered [mg/L]	46	48	18
TOC removal [%]	70	10	14
TN [mg/L]	30	16	18
TN filtered [mg/L]	18	17	18
TN removal [%]	70	10	13
IC [mg/L]	13.2	4.6	18
pH (8:00-9:30 AM)	8.1	1.0	27
DO [mg/L]	7.7	2.4	27
T [°C]	16.3	2.7	27

S1.3 180 L HRAP

S1.3.1 Algal identification

Table 5: Algal identification and abundance for the 180 L HRAP, with values calculated from cell counts L^{-1}. Results were averaged two samples: one preserved in 5% Lugol's iodine, and the second preserved in 10% formaldehyde.

	4-May	**26-May**	**4-Jun**	**15-Jun**	**1-Jul**	**16-Jul**	**27-Jul**	**7-Aug**	**20-Aug**
HRT	7	7	7	7	7	7	4	4	4
Tetracycline in WW	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Mychonastes homosphaera	9.0%								
Scenedesmus quadricauda						3.6%	2.4%		
***Limnothrix* sp.**			9.4%						9.5%
Nitzschia inconspicua	1.8%								
***Thecamoebae* sp. pl.**							1.0%		0.4%
Chrysophyceae						2.8%	2.9%	2.5%	
Desmodesmus armatus	2.1%								
Nitzschia palea	2.1%								
Scenedesmus tenuispina			5.9%	17.1%		3.6%	2.4%		
Nitzschia palea	1.2%								
Chlorella vulgaris	5.5%	3.0%							
***Nitzschia* sp. pl.**			4.1%		1.2%	3.7%	3.0%	10.0%	14.9%
***Vahlkampfia* sp.**				2.9%		1.4%			
Scenedesmus cf. calyptratus	21.3%								
Stigeoclonium tenue	16.0%								
***Microspora* sp.**	19.8%	42.7%	4.1%	8.0%	8.0%				
***Phormidium* sp.**	51.2%								
Acutodesmus obliquus	6.7%	77.1%	81.2%	86.0%	91.4%	91.8%	91.3%	88.7%	80.1%
Ciliata			0.6%						
Egg	1.1%								
Rotifera								0.9%	0.4%
Amoebae sp. pl.	0.6%								

S1.3.2 General monitoring parameters

Table 6: HRAP performances and monitored environmental conditions during stable operation. Data shown as mean ± 95% CI (n).

Stage	I	II	III	IV
Description	Start-up (no TET)	Continuous TET	Continuous TET	Continuous or Pulsed TET
HRT	7	7	4	4
Stable Period	26th May - 16th June	17th June - 21st July	29th July - 6th Aug	6th Aug - 3rd Sep
WW Flow (L/d)	25.5 ± 0.9 (20)	24.1 ± 1.1 (30)	42.9 ± 1.2 (10)	43.6 ± 0.5 (27)
Effluent Flow (L/d)	20.8 ± 5.9 (21)	13.3 ± 1.5 (32)	33.9 ± 5.0 (9)	35.6 ± 2.9 (27)
Average net Evaporation (L/d)	3.5 ± 5.6 (21)	10.0 ± 2.3 (31)	12.4 ± 9.3 (10)	8.0 ± 2.8 (27)
TSS [g/L]: WW	0.12 ± 0.03 (6)	0.13 ± 0.02 (10)	0.11 ± 0.05 (3)	0.12 ± 0.04 (8)
TSS [g/L]: HRAP	1.1 ± 0.3 (6)	1.1 ± 0.1 (10)	1.3 ± 0.6 (3)	1.2 ± 0.1 (8)
TSS [g/L]: Effluent	0.016 ± 0.004 (6)	0.022 ± 0.006 (10)	0.051 ± 0.022 (3)	0.057 ± 0.016 (8)
VSS[1] [g/L]: WW	0.11 ± 0.03 (5)	0.12 ± 0.01 (10)	0.10 ± 0.04 (3)	0.10 ± 0.04 (8)
VSS [g/L]: HRAP	1.0 ± 0.2 (6)	1.0 ± 0.1 (10)	1.1 ± 0.5 (3)	1.0 ± 0.1 (8)
VSS [g/L]: Effluent	0.016 ± 0.005 (5)	0.022 ± 0.006 (10)	0.046 ± 0.010 (3)	0.055 ± 0.015 (8)
VSS [g/L]: Settled biomass	6.2 ± 1.7 (5)	9.2 ± 1.1 (10)	14.3 ± 2.1 (3)	7.0 ± 2.1 (8)
Productivity collected (by settling) [g/m^2/d]	3.9 ± 0.5 (5)	2.6 ± 0.7 (10)	10.5 ± 1.1 (3)	9.9 ± 2.0 (8)
Total productivity [g/m^2/d]	7.4 ± 5.3 (6)	4.5 ± 2.0 (10)	15.0 ± 1.7 (3)	15.9 ± 4.6 (8)
COD [mg/L]: WW filtered	396 ± 112 (6)	373 ± 22 (10)	479 ± 241 (3)	407 ± 31 (8)
COD [mg/L]: Effluent filtered	141 ± 22 (6)	146 ± 12 (10)	145 ± 53 (3)	96 ± 20 (8)
TOC [mg/L]: WW filtered	124 ± 41 (6)	116 ± 8 (9)	126 ± 107 (3)	123 ± 16 (8)
TOC [mg/L]: Effluent filtered	29 ± 2 (6)	37 ± 3 (9)	33 ± 7 (3)	30 ± 4 (8)
TN [mg/L]: WW filtered	89 ± 25 (6)	92 ± 6 (9)	65 ± 23 (3)	80 ± 13 (8)
TN [mg/L]: Effluent filtered	60 ± 6 (6)	86 ± 14 (9)	49 ± 21 (3)	56 ± 10 (8)

[1] Volatile suspended solids

S1.4 Pulse test data monitoring

S1.4.1 Environmental variables recorded during pulse tests

In this section, the data for environmental variables relevant to tetracycline removal are reported. The tetracycline concentrations and sunlight intensities were reported in the main text, and are not repeated here.

Table 7: TSS and VSS concentrations measured over the period of the 900 L HRAP pulse tests to confirm that concentrations were relatively stable.

Name	TSS (g/L)	VSS (g/L)	VSS/TSS
HRAP 8 pm 19th March	0.55	0.43	0.77
HRAP 7 am 20th March	0.53	0.39	0.74
HRAP 9 am 20th March	0.51	0.39	0.76
HRAP 11 am 20th March	0.54	0.41	0.76
HRAP 3 pm 20th March	0.60	0.45	0.76
HRAP 8 pm 24th March	0.54	0.41	0.76
HRAP 7 am 25th March	0.60	0.45	0.75
HRAP 11 am 25th March	0.67	0.49	0.72
HRAP 3 pm 25th March	0.70	0.50	0.71

Table 8: TSS concentrations for pulse tests in the 180 L pilot HRAP

	TSS [g L-1]
7-Aug	1.10
13-Aug	1.36
18-Aug	1.13
20-Aug	1.13
27-Aug	1.15
31-Aug	1.05

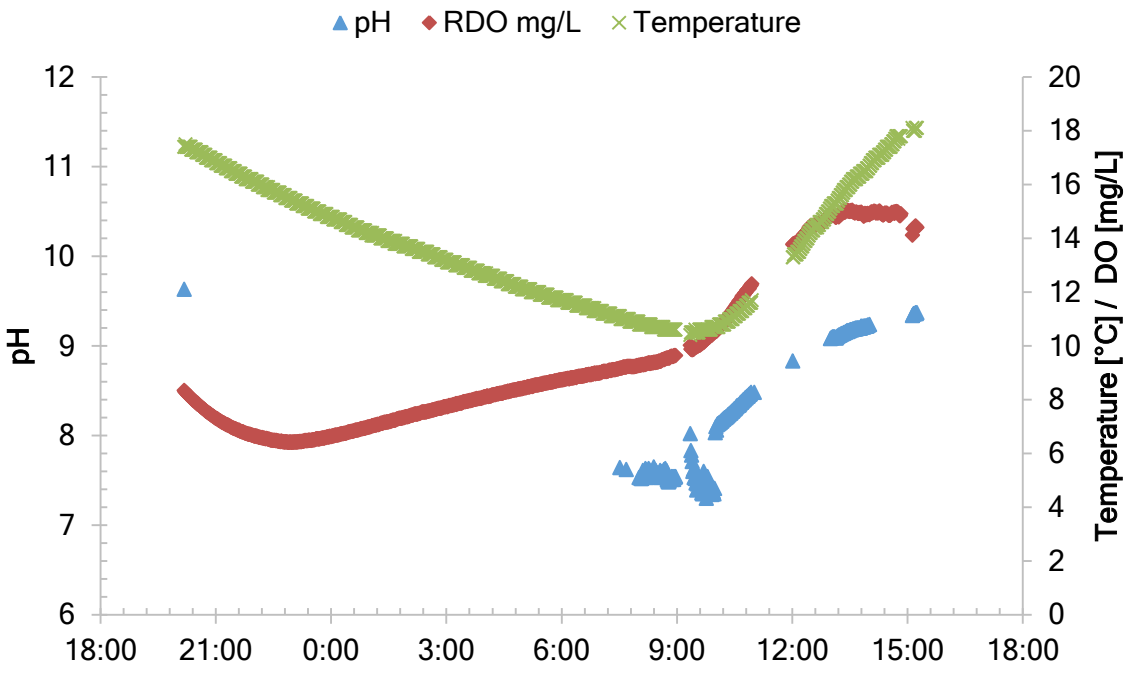

Figure 1: pH, DO, and temperature datalogged 19th-20th March 2015 during the pulse test

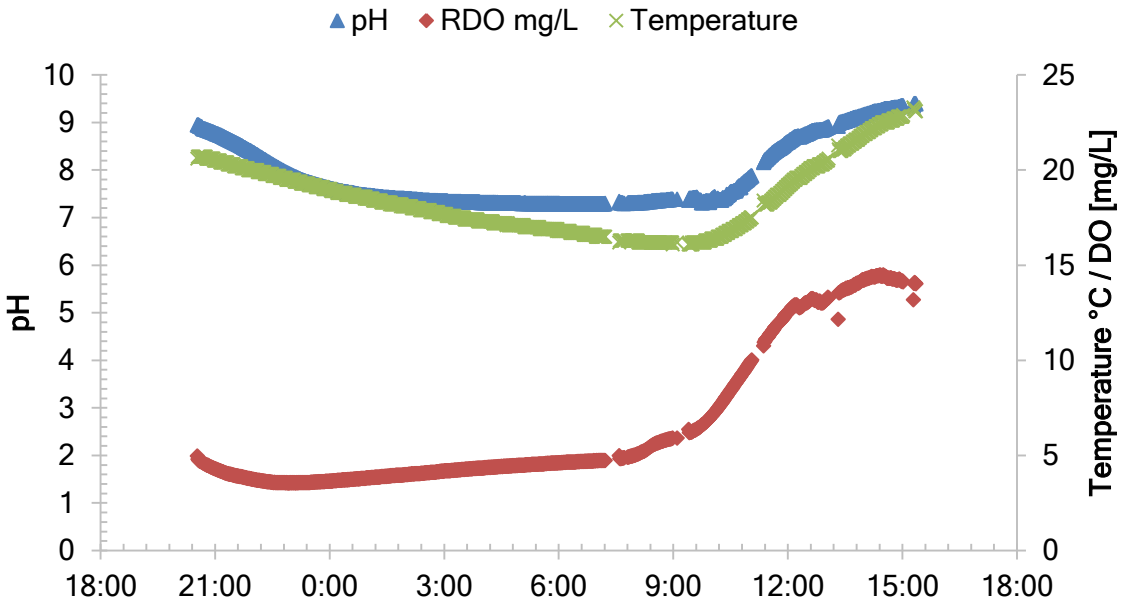

Figure 2: pH, DO, and temperature datalogged 24th -25th March 2015 during the pulse test

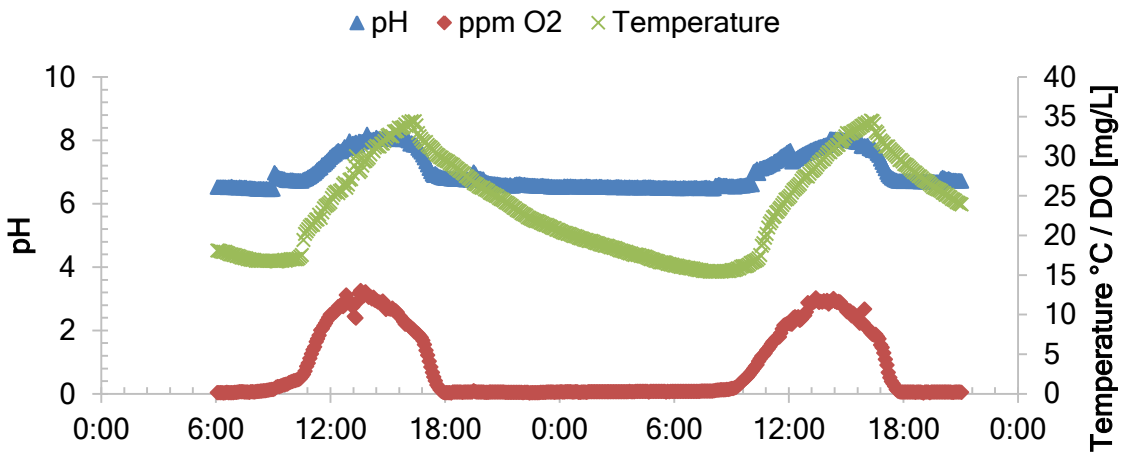

Figure 3: pH, DO, and temperature datalogged 7th -8th Aug 2015 during the pulse test

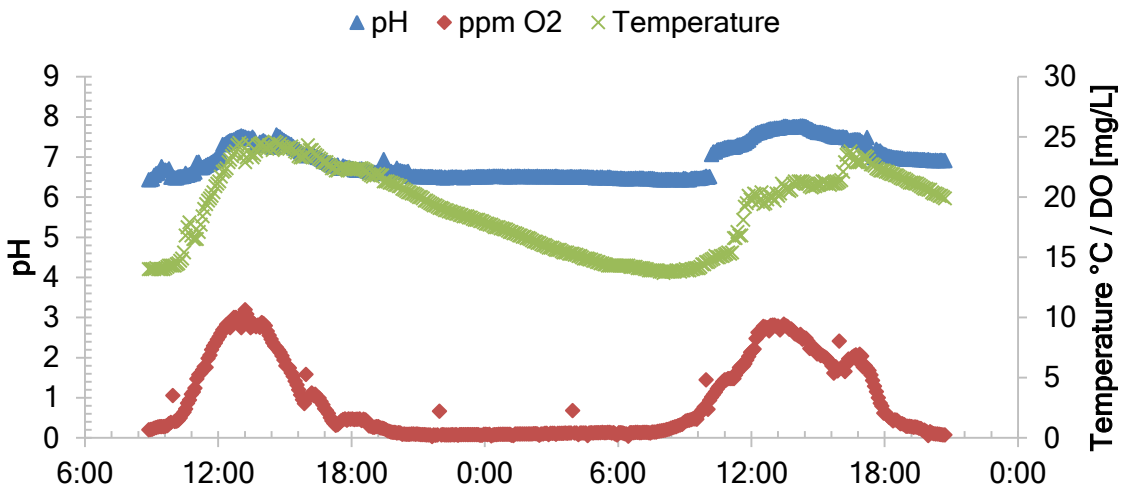

Figure 4: pH, DO, and temperature datalogged 13th -14th Aug 2015 during the pulse test

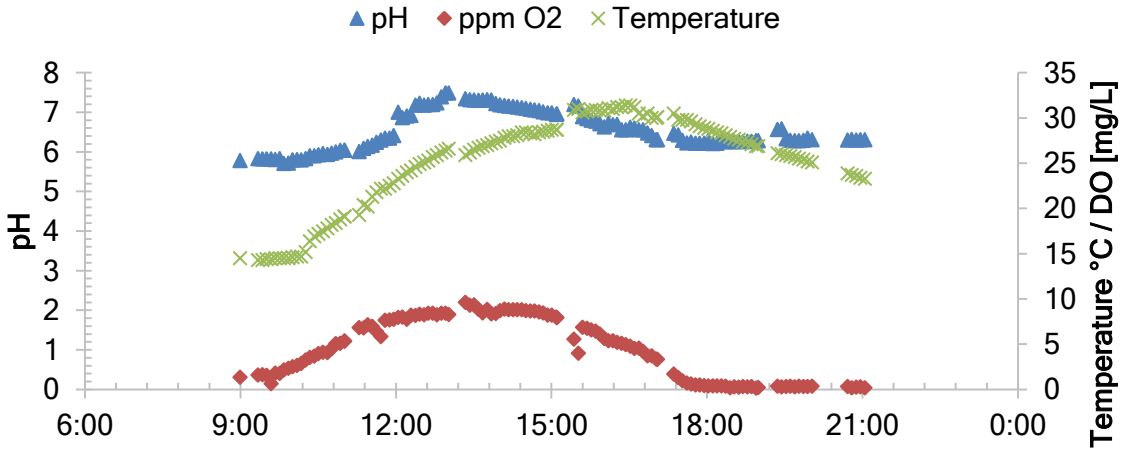

Figure 5: pH, DO, and temperature datalogged 18th Aug 2015 during the pulse test

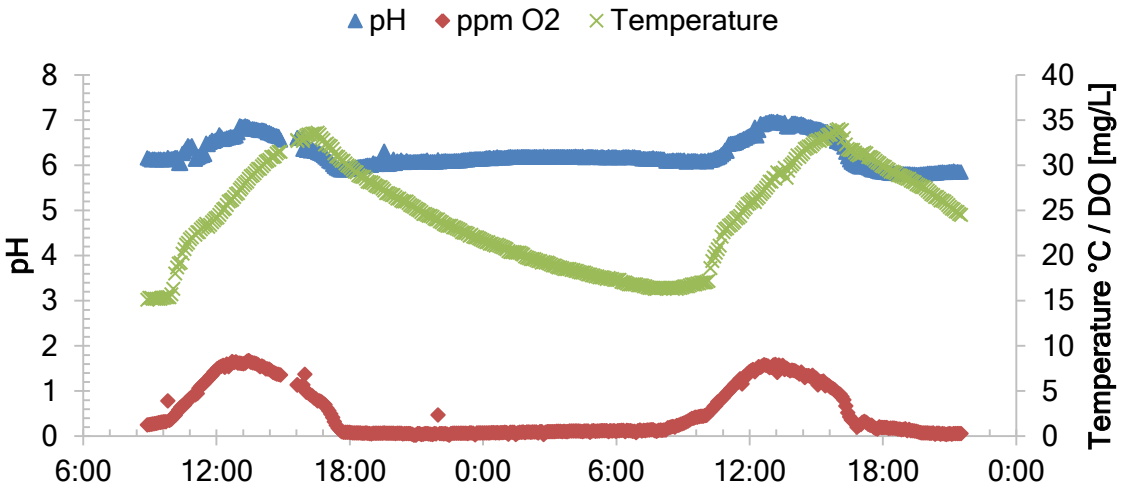

Figure 6: pH, DO, and temperature datalogged 20th -21st Aug 2015 during the pulse test

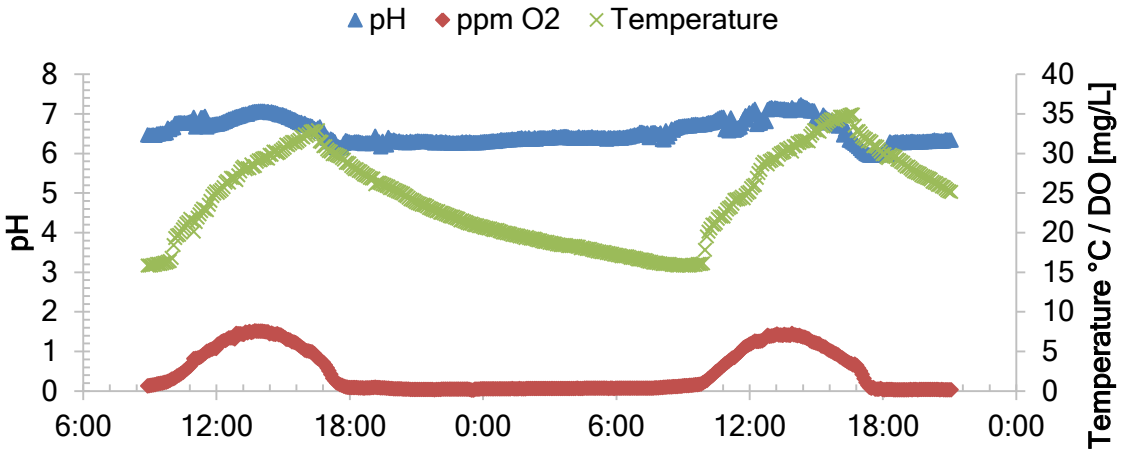

Figure 7: pH, DO, and temperature datalogged 27th -28th Aug 2015 during the pulse test

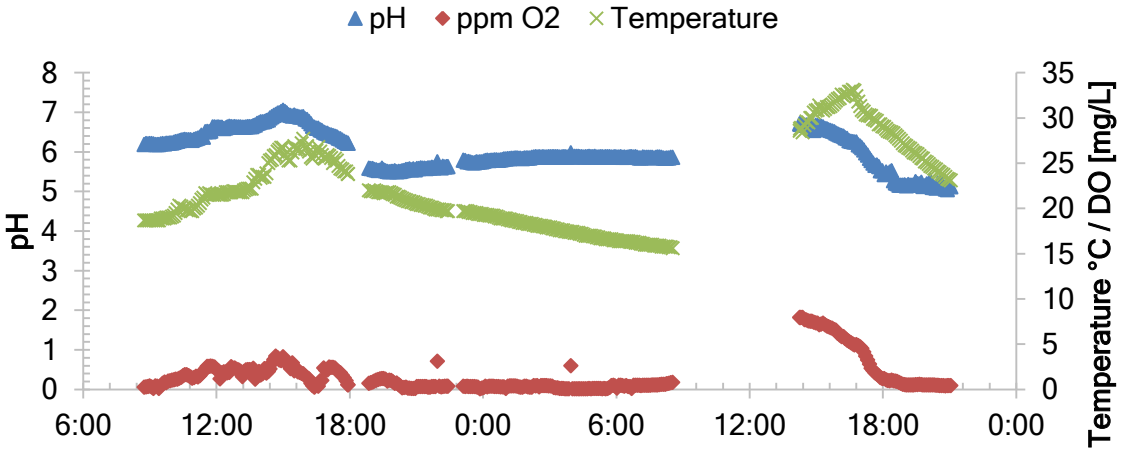

Figure 8: pH, DO, and temperature datalogged 31st– 1st Sep 2015 during the pulse test

S1.4.2 Sorption data during the 180 L HRAP pulse tests

In the sorption extraction results at the end of August, recorded sorbed tetracycline concentrations were unusually high. The problem was not determined, and may be due to a hidden peak underneath the TET peak when integrating the chromatogram (it would have to be a new compound coming through the wastewater), accumulated sorbed TET from previous pulse tests, or could be something else.

This had a minor impact in the batch sorption tests (0.2mg/L to 10 mg/L – giving an intercept for the partition coefficient graph rather than going through zero, and more impact at low ranges). It has a large impact for the pulse tests/HRAP data, meaning that it is difficult to use this data confidently. The effect was not seen in the previous months. Unfortunately, the problem occurred at the end of the research period in Spain, and thus further tests were not possible. The data is presented here in Table 9, as the trends observed in the data are an indication of desorption during the pulse tests, and this is the only data available that does support desorption at the low <100µg/L concentrations in the 180 L HRAP. However, as the mass balance in Table 9 shows, immediately after the TET pulses, the net change in sorbed + aqueous TET concentrations was greater than the TET added to the HRAP. As seen in the table, the sorption recorded usually increases after a pulse and is either stable (overnight) or generally decreases over the daytime after a pulse.

Table 9: Mass balance on sorbed TET during the pulse test series. Four pulse tests are presented in the table, with a blank row between each pulse. Sorbed TET was not measured for the other pulse tests in the pilot HRAPs.

Time	Extracted Sorbed Tetracycline [mg/g_{TSS}]	Extracted Tetracycline per volume of HRAP sample [µg/L]	Aqueous TET concentration [µg/L]	Change in aqueous + sorbed TET conc.[µg/L]	Events	Sorption/Desorption
8/13/2015 10:50	0.032	44	10		**Before pulse**	
8/13/2015 12:00	0.057	77	27	51	1 h after 1st pulse	Expected increase from sorption
8/13/2015 15:30	0.059	79	3	-21	4.5 h after 1st pulse	Slight decrease – not definite desorption
8/13/2015 20:30	0.094	127	87	131	1 h after 2nd Pulse	Expected increase from sorption
8/14/2015 8:00	0.094	128	34	-52	After night	No net change in sorption – low TET removal during the night
8/20/2015 10:25	0.050	57	10		**Before pulse**	
8/20/2015 11:30	0.102	115	61	109	1 h after 1st pulse	By mass balance, increase in sorption is greater than removal of aqueous TET, which is impossible.
8/20/2015 19:00	0.075	84	24	-68	8.5 h after 1st pulse	Clear decrease in sorption
8/20/2015 20:35	0.112	127	82	100	1 h after 2nd Pulse	Clear increase in sorption. Net (sorbed + aqueous) increase in TET concentrations are exactly the increase expected based on the TET pulse added.
8/21/2015 8:15	0.089	101	48	-60	Next day after pulse	Clear decrease in sorption
Time	**Extracted**	**Extracted**	**Aqueous TET**	**Change in**	**Events**	**Sorption/Desorption**

	Sorbed Tetracycline [mg/g_{TSS}]	Tetracycline per volume of HRAP sample [µg/L]	concentration [µg/L]	aqueous + sorbed TET conc. [µg/L]		
8/27/2015 10:30	0.063	73	17		**Before pulse**	**'Before pulse' is getting higher. This corresponds with the aqueous TET conc.**
8/27/2015 10:50	0.050	57	16	-16	**Before pulse**	**Second test 'Before pulse' is a little less – this helps to show the variation in the data, although the sun is up now, so potentially a little desorption**
8/27/2015 12:00	0.075	87	46	59	1 h after 1st pulse	Expected increase in sorption
8/27/2015 18:30	0.071	81	31	-20	8.5 h after 1st pulse	Slight decrease in sorption – possibly due to desorption
8/27/2015 20:10	0.119	137	96	121	1 h after 2nd Pulse	Clear increase in sorption - but net change in aqueous + sorbed TET is again more than the TET pulse added
8/28/2015 8:15	0.124	143	51	-39	Next day after pulse	Slight decrease in sorption overnight this time…
8/31/2015 16:30	0.058	60	27		**Before pulse**	**Sorption 'before' has dropped a bit, although this is a night-only pulse, so the data is after sunlight exposure.**
9/1/2015 10:20	0.122	123	50	85	Overnight after pulse	Clear increase in sorption
9/1/2015 16:20	0.088	89	41	-43	Overnight + daytime after pulse	Clear decrease in sorption after photodegradation.

S1.4.3 Correlation analysis

In order to search for links between the monitored performance and environmental conditions of the HRAPs and the removal of tetracycline, correlations were investigated at different time-steps: 1) correlations with the average tetracycline removal rates compared with average environmental and performance conditions in a steady operating period, and 2) where the data was available, instantaneous degradation rates or rates averaged of 2, 3, 4, or 5 data points were compared with the average environmental or performance conditions in the corresponding time period.

In order to exemplify this second correlation calculation using the example of one of the pulse tests, the original tetracycline data, with data labels naming the points 1-6 during the first pulse degradation during the light period.

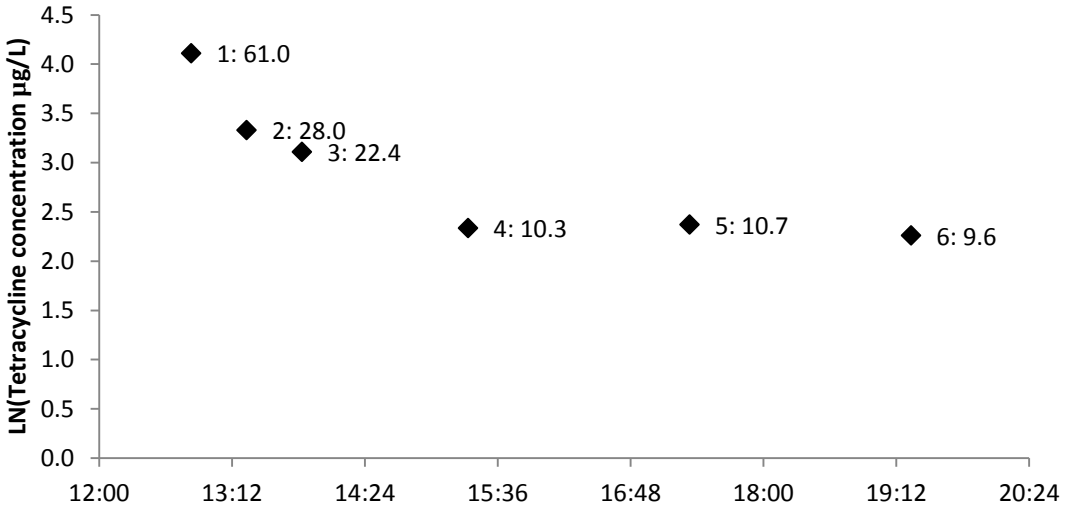

Figure 9: The natural logarithm of tetracycline concentrations during the pulse of tetracycline conducted on the 7th Aug 2015, with points labelled numerically for reference.

The slope of the data in Figure 9 was then calculated between points 1-2, 2-3, 3-4, 4-5, and 5-6, to find the rate of degradation, and correlations and effects plots with the average pH, DO, and temperature values (from datalogging) were checked. Then points

1-3, 2-4, 3-5, and 4-6 were averaged and correlations and effects plots checked; then points 1-4, 2-5, 3-6; then points 1-5, 2-6. The best results were from the averages across four data points[2], so these values were quoted in the thesis. The same method was used to examine 0^{th} order rates, but taking the slope of the TET concentrations, rather than the natural log of the concentrations.

[2] The effects plots showed clustering of values more clearly, and the average of rates across less than 4 data points resulted in some negative TET removal rates – probably due to analytical uncertainty. Therefore the average across the 4 data points minimised this uncertainty and eliminated the negative TET removal rates.

S2. Appendix Two –Indoor tetracycline batch experiments

This section presents the TET monitoring data for all the 100 mL batch experiments conducted in NZ using the biomass sourced from the 7 L HRAPs, including comparisons between the experiments. Although data in the figures is shown with connected lines, it is not meant to imply linear relationships between the points, but simply a guide to help trace the data over time given the large number of data points on some graphs, and the variability observed.

The pseudo-first-order and pseudo-zeroth-order kinetic constants across the different indoor batch experiments are shown in Table 11, to demonstrate that the pseudo-first order kinetic constants provided a better fit to the experimental data than the pseudo-zeroth order kinetic rates. Initial TSS data for the biomass samples are shown in Table 10. pH values are reported in Table 18 to Table 22 (no data on pH was collected for the 9th Sep 2014), and dissolved oxygen concentrations (DO) and temperature values for the final two batch tests are reported in Table 23 and Table 24.

Table 10: Dry weights for biomass sourced from the HRAPs
(initial TSS concentration for samples)

	TSS HRAP A [g/L]	**TSS HRAP B [g/L]**
26/8/2014 9:20	0.54	0.47
9/9/2014 9:20	0.22	0.10
17/10/2014 9:20	0.27	0.33
1/12/2014 9:20	0.07	0.08
15/12/2014 9:20	0.16	0.23

Table 11: Averaged kinetic constants and R^2 values for the batch tests

	0th order	**1st order**
	k value (R^2)	k value (R^2)
Units	mg $L^{-1}d^{-1}$	d^{-1}
2nd Sep Batch biomass test 50% wwater & light (with and without algae)	0.031 (0.75)	0.94 (0.89)
2nd Sep Batch biomass test 50% wwater & dark	0.011 (0.49)	0.21 (0.51)
26th Aug Std Batch Light Algae Samples	0.046 (0.93)	2.31 (0.99)
26th Aug Std Batch Dark Algae Samples	0.005 (0.92)	0.12 (0.97)
9th Sep Std Batch Light Algae Samples	0.061 (0.81)	3.00 (1.00)
9th Sep Std Batch Dark Algae Samples	0.003 (0.85)	0.05 (0.88)
17th Oct Std Batch Light Algae Samples	0.018 (0.66)	1.33 (0.95)
17th Oct Std Batch Dark Algae Samples	0.006 (0.88)	0.12 (0.95)
1st Dec 2014 Light Algae Samples Active	0.031 (0.79)	0.82 (0.92)
1st Dec 2014 Dark Algae Samples	0.004 (0.51)	0.08 (0.53)
1st Dec 2014 Light Algae Samples Autoclaved	0.030 (0.78)	1.45 (0.96)
15th Dec 2014 Light Algae Samples	0.022 (0.65)	2.26 (0.96)
15th Dec 2014 Dark Algae Samples	0.010 (0.83)	0.30 (0.92)

Table 12: Tetracycline concentrations for the batch test 2nd Sep 2014

Sampling time (h)	**0**	**1**	**4**	**8**	**24**
0.1 g/L Pond A	1.5	1.1	1.0	0.8	0.5
0.1 g/L Pond A'	1.5	1.1	1.0	0.8	0.5
0.25 g/L Pond A	1.5	1.0	0.9	0.8	0.5
0.25 g/L Pond A'	1.5	1.0	0.9	0.8	0.5
0.5 g/L Pond A	1.5	1.0	0.9	0.8	0.5
0.5 g/L Pond A'	1.4	1.0	0.9	0.7	0.5
0.25 g/L Pond B	1.0	1.4	0.9	0.8	0.4
0.25 g/L Pond B'	1.4	1.0	0.8	0.6	0.4
50% wwater	1.6	0.9	0.9	0.7	0.4
50% wwater'	1.5	0.9	0.9	0.8	0.5
50% wwater dark	1.5	0.9	1.2	1.2	1.1
MQ water	1.8	0.9	1.9	1.7	1.0

Table 13: Tetracycline concentrations for the batch test 29th Aug 2014

Sampling time (h)	0	4	8	24	72	144
MQ water, Dark	2.0			2.0	2.0	
Pond A, Dark, Autoclaved	2.0			1.1	0.8	0.6
Pond B, Dark, Autoclaved	2.0			1.1	0.7	0.5
Pond A, Dark, Active	2.0			1.1	0.9	0.7
Pond B, Dark, Active	2.0			1.2	0.9	0.7
MQ water, Light	2.0		2.0	1.8	1.3	
Pond A, Light, Autoclaved	2.0	0.5	0.6	0.0		
Pond B, Light, Autoclaved	2.0	0.6	0.4	0.1		
Pond A, Light, Active	2.0	1.1	0.8	0.3		
Pond B, Light, Active	2.0	2.2	0.7	0.1		

Table 14: Tetracycline concentrations for the batch test 9th Sep 2014

Sampling time (h)	0	4.5	8	25	72	144
Pond A, Light, Active	2.0	1.2	0.7	0.2		
Pond A, Light, Autoclaved	1.5	0.6	0.4	0.0		
Pond B, Light, Active	2.1	1.1	0.9	0.1		
Pond B, Light, Autoclaved	1.6	0.9	0.6	0.0		
MQ water, Light	1.9		1.8	1.6	1.4	0.8
Pond A, Dark, Active				1.5	1.4	1.3
Pond A, Dark, Autoclaved	1.6			1.1	1.2	1.1
Pond B, Dark, Active	2.0			1.6	1.4	1.2
Pond B, Dark, Autoclaved	1.7			1.3	1.3	1.0
MQ water, Dark	1.8			1.6	1.6	1.7

Table 15: Tetracycline concentrations for the batch test 17th Oct 2014

Sampling time (h)	0	1	4	8	24	72	144
HRAP-A, Light, Active	1.9	1.5	1.0	0.6	0.1	0.0	
HRAP-A, Light, Autoclaved	1.2	0.9	1.0	0.6	0.0	0.0	
HRAP-B, Light, Active	1.8	1.5	0.9	0.8	0.3	0.0	
HRAP-B, Light, Autoclaved	1.3	1.4	1.0	0.8	0.2	0.0	
HRAP-A, Dark, Active	1.8	1.8		1.5	1.4	1.0	0.7
HRAP-A, Dark, Autoclaved	1.8	2.2		2.7	2.3	1.5	0.5
HRAP-B, Dark, Active	1.9	1.8		1.5	1.4	0.9	1.3
HRAP-B, Dark, Autoclaved	1.3	1.4		1.2	1.0	0.8	0.5
MQ water, Light	1.8	1.9	2.0	1.7	1.7	1.2	
MQ water, Dark	1.8	1.8		1.9	1.7	1.5	1.5
pH 8 PO4 buffer, Light	1.9	1.9	1.7	1.6	0.4	0.3	
pH 8 PO4 buffer, Dark	2.0	2.0		1.8	1.8	1.9	1.2
pH 7 PO4 buffer, Light	2.0	1.8	1.4	1.0	1.1	0.1	
pH 7 PO4 buffer, Dark	1.9	1.8		1.9	1.8	1.7	1.5
Filtrate, Light	2.1	1.9	1.6	1.3	0.6	0.1	

Table 16: Tetracycline concentrations for the batch test 1st Dec 2014

Sampling time (h)	0	4	8	24	45	72	168
HRAP-A, Light, Active	1.9	1.4	1.0	0.6	0.5		
HRAP-B, Light, Active	2.1	1.6	1.1	0.5	0.3		
HRAP-A, Light, Autoclaved	1.6	1.1	0.7	0.2	0.1		
HRAP-B, Light, Autoclaved	1.7	1.1	0.8	0.3	0.1		
HRAP-A, Dark, Active	1.6			1.1		0.8	1.5
HRAP-B, Dark, Active	2.0			1.0		0.9	0.5
HRAP-A, Dark, Autoclaved	1.6			1.1		0.9	1.2
HRAP-B, Dark, Autoclaved	1.0			0.9		0.8	0.4
pH 7 PO4 buffer, Light	1.9		1.7	1.7	0.8		
pH 7 PO4 buffer, Dark	1.6			1.8		1.7	0.9
MQ water, Light	1.6		1.8	1.6	1.3		
MQ water, Dark	1.7			1.2		1.6	0.9

Table 17: Tetracycline concentrations for the batch test 15th Dec 2014

Sampling time (h)	0	4	8	24	48	72
HRAP-A, Light, Active	1.4	0.6	0.3	0.0	0.0	
HRAP-B, Light, Active	1.4	0.7	0.4	0.1	0.0	
HRAP-A, Light, Autoclaved	1.4	0.7	0.6	0.0	0.0	
HRAP-B, Light, Autoclaved	1.1	0.6	0.5	0.1	0.0	
HRAP-A, Dark, Active	1.4		1.0	0.7	0.6	0.5
HRAP-B, Dark, Active	1.3		1.0	0.9	0.7	0.5
HRAP-A, Dark, Autoclaved	1.3		1.1	0.7	0.6	0.5
HRAP-B, Dark, Autoclaved	1.1		0.7	0.6	0.5	0.4
pH 7 PO4 buffer, Light	1.7		1.4	0.9	0.6	0.4
pH 7 PO4 buffer, Dark	1.7		1.6	1.5	1.3	1.5
MQ water, Light	1.4		1.4	1.2	1.0	0.8
MQ water, Dark	1.5		1.3	1.1	1.1	1.2

Table 18: Final pH values for the batch test begun 26th Aug 2014.

	final pH
Pond A, Light, Active	9.87
Pond A, Light, Autoclaved	8.17
Pond B, Light, Active	10.01
Pond B, Light, Autoclaved	8.15
MQ water, Light	7
Pond A, Dark, Active	7.85
Pond A, Dark, Autoclaved	8.36
Pond B, Dark, Active	7.94
Pond B, Dark, Autoclaved	8.33
MQ water, Dark	6.97

Table 19: Final pH values for the batch test begun 2nd Sept 2014

	final pH
0.1 g/L Pond A	8.57
0.1 g/L Pond A'	8.55
0.25 g/L Pond A	8.99
0.25 g/L Pond A'	8.79
0.5 g/L Pond A	8.97
0.5 g/L Pond A'	8.9
0.25 g/L Pond B	8.94
0.25 g/L Pond B'	8.97
50% wwater	8.34
50% wwater'	8.32
50% wwater dark	8.39
MQ water	7.82

Table 20: Initial pH values and final pH values for the dark samples of the batch test begun 17th Oct 2014. The final pH values of the light samples were not recorded

	pH initial	pH final
HRAP-A, Light, Active	7.66	
HRAP-A, Light, Autoclaved	9.18	
HRAP-B, Light, Active	7.25	
HRAP-B, Light, Autoclaved	8.71	
Filtrate, Light	7.46	
pH 7 PO4 buffer, Light	7	
pH 8 PO4 buffer, Light	8	
HRAP-A, Dark, Active	7.66	6.09
HRAP-A, Dark, Autoclaved	9.18	6.01
HRAP-B, Dark, Active	7.25	8.22
HRAP-B, Dark, Autoclaved	8.71	8.06
MQ water, Dark		7.1
pH 7 PO4 buffer, Dark	7	7.18
pH 8 PO4 buffer, Dark	8	8

Table 21: pH values during batch experiment 1st Dec 2014

Sampling time (h)	0	24	45	168
HRAP-A, Light, Active	6.75	6.2	6.18	
HRAP-B, Light, Active	6.79	7.05	6.91	
HRAP-A, Light, Autoclaved	6.88	6.7	6.96	
HRAP-B, Light, Autoclaved	7.24	6.86	7.04	
HRAP-A, Dark, Active	6.74	6.44		5.33
HRAP-B, Dark, Active	6.8	6.82		5.32
HRAP-A, Dark, Autoclaved	6.86	6.61		7.25
HRAP-B, Dark, Autoclaved	7.24	6.798		6.98
pH 7 PO4 buffer, Light	7.07	7.04	7.03	
pH 7 PO4 buffer, Dark	7.06	7.04		7.09
MQ water, Light	7.7	7.51	6.69	
MQ water, Dark	7.4	7.54		7.38

Table 22: pH values during batch experiment 15th Dec 2014

Sampling time (h)	0	24	48	72
HRAP-A, Light, Active	7.79	10.49	10.84	
HRAP-B, Light, Active	7.76	9.95	10.42	
HRAP-A, Light, Autoclaved	7.42	7.51	7.87	
HRAP-B, Light, Autoclaved	8.36	7.47	7.87	
HRAP-A, Dark, Active	7.67	7.54		7.55
HRAP-B, Dark, Active	7.77	7.56		7.61
HRAP-A, Dark, Autoclaved	7.4	7.74		8
HRAP-B, Dark, Autoclaved	8.34	7.72		7.83
pH 7 PO4 buffer, Light	7.09	7.1		7.09
pH 7 PO4 buffer, Dark	7.09	7.1		7.12
MQ water, Light	7.92	7.83		6.93
MQ water, Dark	8.22	7.53		6.44

Table 23: DO[a] and temperature during batch experiment 15th Dec 2014

	DO values [mg/L]			Temperature [°C]		
Sampling time (h)	24	48	72	24	48	72
HRAP-A, Light, Active	9.1	8.81		23.8	22.9	
HRAP-B, Light, Active	8.39	8.49		23.9	22.9	
HRAP-A, Light, Autoclaved	7.99	7.66		23.5	22.6	
HRAP-B, Light, Autoclaved	8.28	7.7		23	22	
HRAP-A, Dark, Active	8.13		8.09	23.4		24
HRAP-B, Dark, Active	8.21		8.15	23		23.8
HRAP-A, Dark, Autoclaved	7.82		7.61	24.1		24.9
HRAP-B, Dark, Autoclaved	7.97		7.93	23.6		24.2
pH 7 PO4 buffer, Light	8.53		8.5	23.1		24.1
pH 7 PO4 buffer, Dark	8.34		8.23	23.70		23.6
MQ water, Light	8.48		8.24	23.20		24.6
MQ water, Dark	8.29		8.11	23.90		23.8

[a] Dissolved oxygen concentrations

Table 24: DO and temperature during batch experiment 1st Dec 2014

	DO values [mg/L]			Temperature [°C]		
Sampling time (h)	**24**	**45**	**168**	**24**	**45**	**168**
HRAP-A, Light, Active	8.28	8.35		24.4	24.3	
HRAP-B, Light, Active	8.23	8.53		24.4	23.9	
HRAP-A, Light, Autoclaved	8.01	8.22		24.9	24.1	
HRAP-B, Light, Autoclaved	8.13	8.17		24.4	24.1	
HRAP-A, Dark, Active	8		8.47	23.7		22.5
HRAP-B, Dark, Active	8.16		8.45	23.2		22.2
HRAP-A, Dark, Autoclaved	7.92		8.07	24.5		23.1
HRAP-B, Dark, Autoclaved	7.97		8.19	24.4		22.9
pH 7 PO4 buffer, Light	8.22	8.23		24.2	24	
pH 7 PO4 buffer, Dark	8.65		8.23	22.50		22.1
MQ water, Light	8.23	8.21		24.40	24.3	
MQ water, Dark	8.10		8	24.50		23

S3. Appendix Three – All 2.5 L pot batch experiments

In this appendix, all of the batch experiments for the 2.5 L pots are reported, including the data already presented in the thesis, so that the results can be seen together. Comments on some of the graphs are included in figure captions. pH, DO, T, and PAR light are shown as relevant, along with the aqueous tetracycline concentrations and sorbed tetracycline extraction results. During the pH tests for the antibiotic mixtures with four other antibiotics, sorption extraction tests were performed in case the method was relevant to the other antibiotics also, but the controls showed very poor recovery and hence the results were not considered useful. TSS for all experiments, where available, are shown in Table 25 and Table 26, and the final volumes of the full-day batch experiments are shown in Table 27, noting that some volume was reduced due to sampling, but there was significant evaporation during the day.

In addition, the first sub-section (Section S3.1) discusses a 'matrix effect' observed in a few experiments, where the initial TET concentration supplied was not detected at the initial sampling point, even with clarified effluent samples (negligible TSS concentrations). A pretreatment of the HPLC samples with citric acid + EDTA addition to the HPLC vials was trialled, and this pretreatment did improve recovery of the initial TET concentration in some cases. In addition, full recovery of TET in MQ water samples incubated in the dark was consistently obtained after the citric acid + EDTA pretreatment. This 'matrix effect' was not discussed in the main thesis as the cause is still uncertain, and the results do not change the conclusions for the experiments discussed in the thesis. Therefore, it was deemed a distraction from the main line of argument in the thesis, and is therefore discussed here in the appendices.

Three of the full-day batch tests (5th Aug, 17th Aug, 24th Aug) include results for antibiotic concentrations recorded for a sample of '10% HRAP in MQ', which was not included in the results and discussion of the main text of the thesis, to prevent confusion between this sample and the '10% HRAP in Effleunt' sample. For each batch tests, these two samples were prepared in 2.5 L pots, with 250 mL of HRAP biomass/WW taken directly from the HRAP, and then '10% HRAP in MQ' sample was diluted with 2.2 L of MQ water, and the '10% HRAP in Effluent' sample was diluted with 2.2 L of clarified effluent. Finally, 50 mL of TET stock solution was added to make a total volume of 2.5 L. The purpose of the '10% HRAP in MQ' samples was to assess the impact of dissolved organics upon antibiotic photodegradation, as dissolved organics are involved with the indirect photodegradation pathway. By comparison to the '10% HRAP sample, which had the same concentration of HRAP biomass, but more dissolved organics from the clarified effluent, the effect of dissolved organics on the photodegradation processes might be observed.

Table 25: Original TSS values for the first two full-day batch tests (final TSS not recorded)

28 May 2015	**Original TSS of biomass from HRAP[g/L]**	**1 June 2015**	**Original TSS of biomass from HRAP[g/L]**
ActiveA (interrupted mixing)	1.27	ActiveA (interrupted mixing)	1.33
ActiveB	1.27	ActiveB	1.33
Dead/Autoclaved	1.27	Dead/Autoclaved	1.33

Table 26: Final TSS values recorded for batch experiments in 2.5 L pots.

Date/Sample	Final TSS [g/L]	Date/Sample	Final TSS [g/L]
Full-day batch tests:			
9 Jul 2015		6 Aug 2015	
HRAP 100%	1.43	100% HRAP	1.46
HRAP 67%	0.99	10% HRAP in effluent	0.27
HRAP 33%	0.55	Autoclaved	1.15
Autoclaved	1.35	10%HRAP in MQ	0.15
Filtrate	0.01	Filtrate	0.07
17 Aug 2015		25 Aug 2015	
100% HRAP	1.10	100% HRAP	1.13
10% HRAP in effluent	0.27	10% HRAP in effluent	0.22
Autoclaved	0.90	Autoclaved	0.85
10%HRAP in MQ	0.10	10%HRAP in MQ	0.11
Filtrate	0.06	Filtrate	0.04
HRAP			
pH batch tests			
4 Aug 2015			
unadjusted pH	1.03		
6 pH	0.97		
8.5 pH	0.98		
10.5 pH	0.96		
21 Aug 2015		28 Aug 2015	
N	0.99	N	1.08
5.5pH	0.93	5.5pH	1.05
8.5pH	0.96	8.5pH	0.98
10.5pH	1.06	10.5pH	0.99
Sorption batch tests			
22 Jun 2015		**1 Sep 2015**	
Active 100%HRAP	0.97	0.2 mg/L TET	0.95
Active 67%HRAP	0.56	0.5 mg/L TET	0.96
Active 33%HRAP	0.31	1.0 mg/L TET	0.93
Dead 100%HRAP	0.71	2.0 mg/L TET	0.93
Dead33%HRAP	0.26	5.0 mg/L TET	0.97
MQ	0.00	10 mg/L TET	0.96

Table 27: Final volumes recorded for full-day batch experiments in 2.5 L pots. Final volume decreases from the original 2.5 L due to the 10 mL samplings taken for TET quantification, and evaporation

Date/Sample	**Final Volume [L]**	**Date/Sample**	**Final Volume [L]**
28 May 2015		**1 June 2015**	
ActiveA (interrupted mixing)	2.26	ActiveA (interrupted mixing)	2.28
ActiveB	2.27	ActiveB	2.33
Dead/Autoclaved	2.25	Dead/Autoclaved	2.3
ControlMQ	2.3	ControlMQ	2.35
9 Jul 2015		**6 Aug 2015**	
HRAP 100%	2.12	100% HRAP	2.17
HRAP 67%	2.13	10% HRAP in effluent	2.21
HRAP 33%	2.18	MQ water	2.2
Autoclaved	2.08	Autoclaved	2.3
MQ water	2.11	10%HRAP in MQ	2.19
Filtrate	2.18	Filtrate	2.24
17 Aug 2015		**25 Aug 2015**	
100% HRAP	2.2	100% HRAP	2.23
10% HRAP in effluent	2.24	10% HRAP in effluent	2.2
MQ water	2.22	MQ water	2.25
Autoclaved	2.21	Autoclaved	2.2
10%HRAP in MQ	2.23	10%HRAP in MQ	2.24
Filtrate	2.50	Filtrate	2.23

S3.1 'Matrix effects' and reversible hydrolysis observed during batch tests

In this section, the 'matrix effect' is discussed which was observed in a few batch experiments which used biomass from the 180 L pilot HRAP in Spain. In these experiments, the initial TET concentration supplied was not detected at the initial sampling point, even with filtered effluent samples (negligible TSS concentrations). Since it was thought that a contaminant in the wastewater was complexing with the TET, and thus preventing its detection on the HPLC, this drop in initial TET concentrations was referred to as a 'matrix effect'.

The strongest 'matrix effect' was observed in July, at the end of the 7-day HRT operation of the 180 L pilot HRAP. A full day batch test was conducted in July (Figure 10). In this test, the initial tetracycline concentration should have been 2 mg L^{-1}, the usual concentration used for batch tests in this thesis, but for all samples except the MQ water control, detection of TET was always below 0.5 mg L^{-1}. The MQ water control demonstrated that the stock solution was not the cause of the low measured concentrations. It was also noted that lower concentrations of biomass were more affected than high concentrations, which lead to the hypothesis that the low concentrations were caused by something in the wastewater itself, and not unusually high sorption.

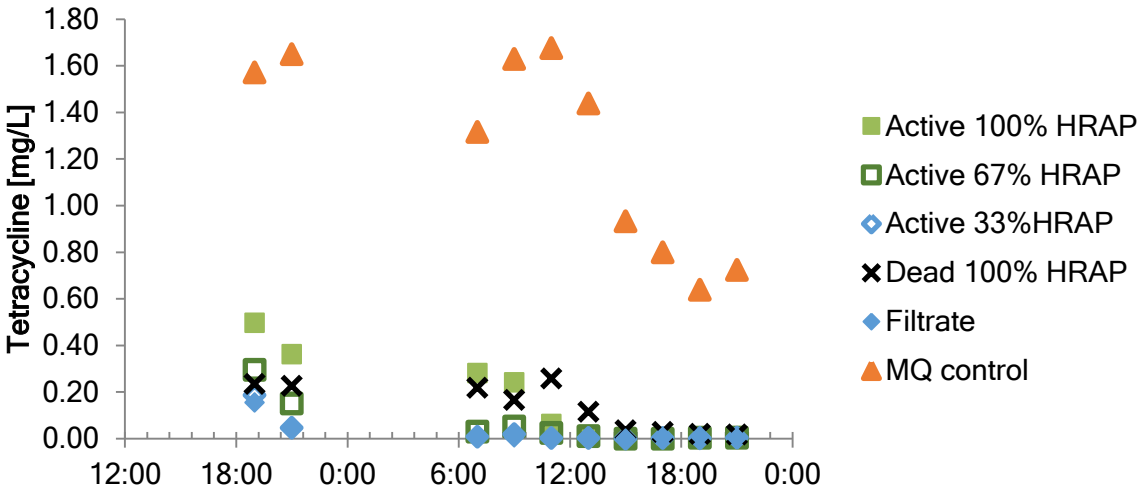

Figure 10: Tetracycline concentrations during full-day batch test 9th-10th Jul 2015

In order to confirm this hypothesis that there was a matrix effect of the wastewater, an experiment was conducted (before any operational changes were made to the HRAP), studying different dilutions of clarified HRAP effluent with MQ water. In addition, filtered samples for HPLC measurement were processed normally, and a second (1 mL) sample was taken, with aliquots of citric acid (0.2 mL, 0.1 M) and Na_2EDTA solution (5%, 0.01 mL) in order to buffer and stabilize the sample. As shown in Figure 11, the 'matrix effect' is clearly linked with the concentration of HRAP effluent. Pretreatment with 3 g of activated-carbon per L of effluent and then filtration (to discount any reactive oxygen species or unusually reactive dissolved organics) only reduced the 'matrix effect' slightly – about the same as a 50% dilution with MQ water. Na_2EDTA by itself did not affect the results (data not presented), but the combination of citric acid with Na_2EDTA did show significant decrease in the 'matrix effect' observed. Citric acid was not tested independently of the Na_2EDTA in this experiment, so subsequent experiments used the combination of both citric acid and Na_2EDTA addition to keep effects consistent, although it is probable that the primary effect was due to the citric acid. Overall, the results suggest that the tetracycline has not been taken out of the solution, but that there is some conversion to another form (perhaps to an epimer, or

conjugated form) that can reverses and thus returns the parent tetracycline molecule when the solution is pretreated with citric acid + EDTA.

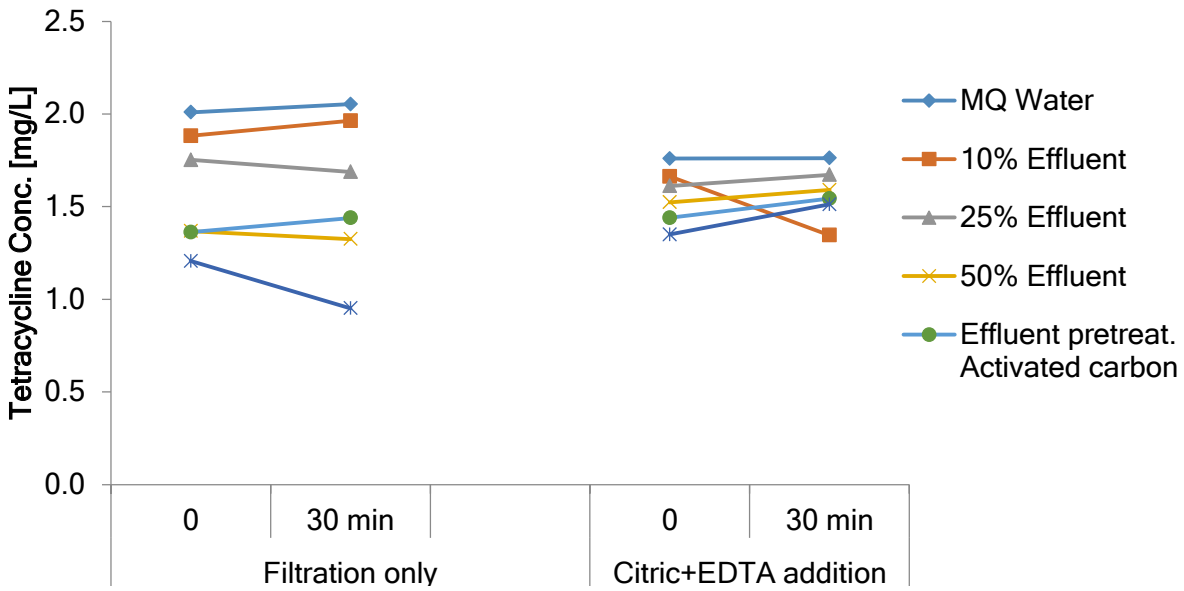

Figure 11: The matrix effect of tetracycline added to HRAP effluent demonstrated through dilutions with MQ water, and the diminishing of this effect when citric acid is added to the HPLC vials. Samples were taken during HRAP operation with 7 days HRT, just before changing to 4 days HRT.

The observed matrix effect was either very inconsistent, or the change of the HRAP operation to 4 days HRT influenced the matrix effect. When the test with MQ water dilutions was repeated after 2 months of HRAP operation at 4 days HRT, the matrix effect observed changed. This time the MQ water dilution made little difference to the samples without citric acid + EDTA addition (Figure 12), but with the citric acid + EDTA addition the samples that were more diluted with MQ water had better recovery of the tetracycline concentration. Therefore the matrix effect during 4 day HRT operation had a greater effect on the detection of the tetracycline than before, and even

small amounts of the effluent WW still has a strong effect in lowering the detected tetracycline concentration. Based on the inconsistency in the effects of the 'matrix effect' and the change in results for the second 'matrix batch test' in Figure 12, we suggest that the 'matrix effect' was indeed caused by some component of the HRAP effluent, but unfortunately we were unable to identify the cause. Because of this observed difference in measurement results with citric acid + EDTA addition to the HPLC samples, all further experiments were conducted with both direct filtration and with citric acid and EDTA addition after filtration. This enabled comparison of the two results, in order to gain further insight into the matrix effect.

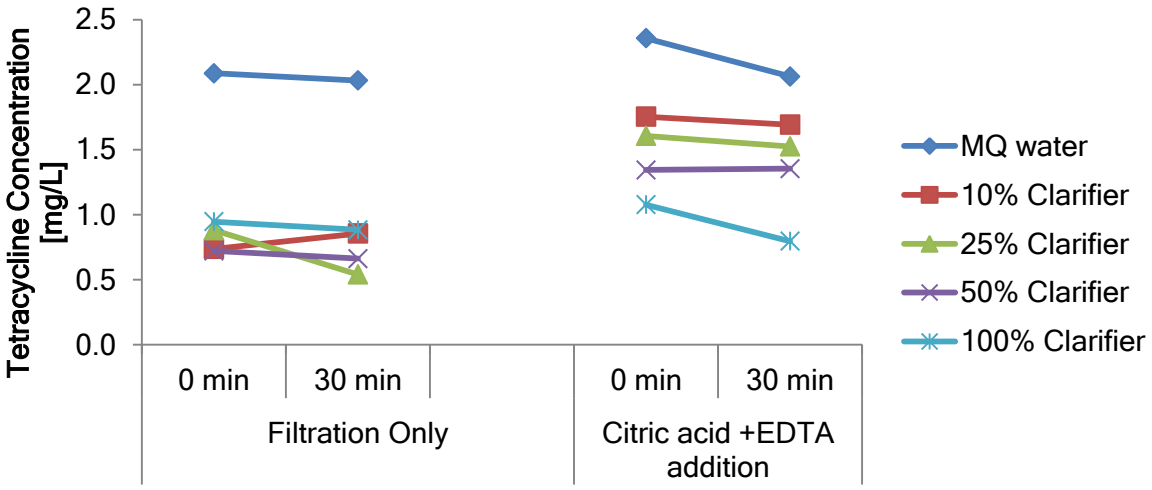

Figure 12: The matrix effect of tetracycline added to HRAP effluent demonstrated through dilutions with MQ water, and the diminishing of this effect when citric acid is added to the HPLC vials. Samples were taken during HRAP operation with 4 days HRT, during the pulse experiments in September.

In order to illustrate the impact of this 'matrix effect' and its impact upon the TET removal in the batch tests, results for the full-day outdoor batch test on the 5th Aug 2015 are shown in Figure 13, with results both with and without the citric acid + EDTA

pretreatment. The most obvious change is for the 'MQ water' sample, and the '10% HRAP in MQ water' sample. For both of these samples, without the pretreatment, the detected TET concentrations decreased during the night. However, with the pretreatment, the TET concentrations in the 'MQ water sample were stable at the initial TET concentration, confirming that no irreversible abiotic degradation (such as hydrolysis) was occurring in these experiments. Also with the pretreatment, the TET concentrations for the '10% HRAP in MQ' sample decreased slightly and then remained steady during the night, with the small decrease expected based upon the ~0.1 g L^{-1} TSS concentration in this sample. For all the other samples with HRAP biomass and/or HRAP effluent the citric acid + EDTA pretreatment made little change to the results.

This 'matrix effect' may explain the similar initial decrease in TET seen for the effluent filtrate sample during the night (~0 g L^{-1} TSS), compared with the 100% HRAP sample (~1.1 g L^{-1} TSS), despite the fact that sorption was found to be the main removal mechanism in the dark. Some sorption may still be possible due to dissolved organics[a] (especially extracellular proteins, as TET tends to sorb to proteins rather than carbohydrates(Song et al., 2014)). However, it is unlikely that the same sorption could occur to the dissolved organics in the clarifier filtrate compared with the 100% HRAP sample, therefore the decrease in TET concentrations during the night is most likely due to this 'matrix effect'. The results in the main thesis text, Section 4.4.3, still confirm that sorption was the main removal mechanism in the dark, as sorbed TET was confirmed by sorption extraction.

[a] Especially extracellular proteins, as TET tends to sorb to proteins rather than carbohydrates; Song, C., Sun, X.F., Xing, S.F., Xia, P.F., Shi, Y.J., Wang, S.G., 2014. Characterization of the interactions between tetracycline antibiotics and microbial extracellular polymeric substances with spectroscopic approaches. Environ. Sci. Pollut. Res. 21, 1786–1795.

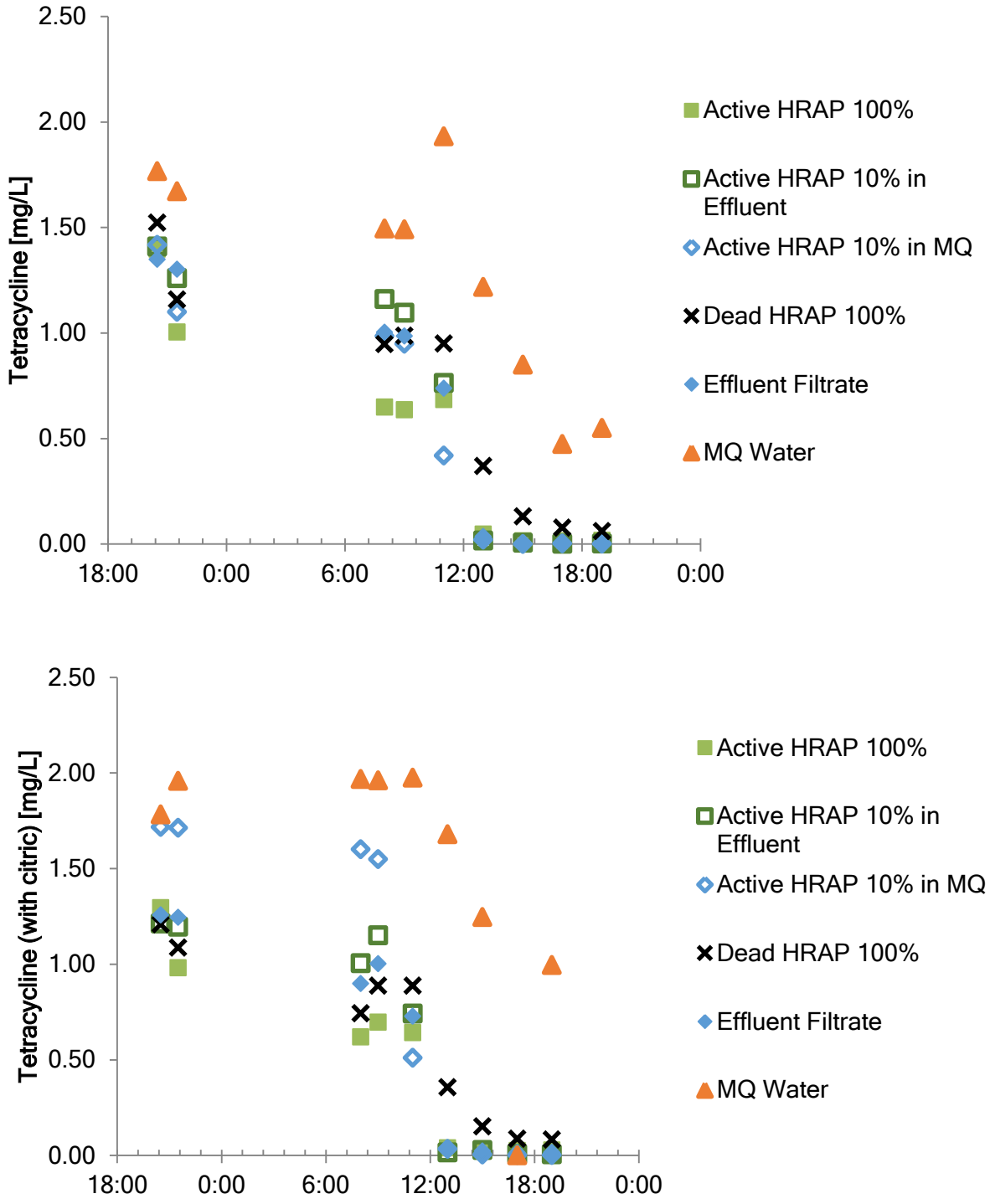

Figure 13: Tetracycline concentrations with or without citric acid + EDTA pretreatment to HPLC samples for the full day batch test 5th-6th Aug

Therefore, to conclude for this sub-section:

- Pretreatment with citric acid + EDTA consistently increased the detected TET concentrations in MQ water samples under dark conditions, demonstrating that no irreversible abiotic TET degradation occurred in the MQ water control samples in the dark.
- In HRAP effluent samples during July and Aug, the initial TET concentrations in batch tests were reduced due to an unknown effect, termed a ‘matrix effect’, which could be due to some component of the HRAP effluent conjugating with the TET, or triggering a rapid epimerisation of TET to an undetected form. Pretreatment of HPLC samples with citric acid + EDTA reduced this ‘matrix effect’ for some batch experiments. However, based on the results obtained, the main conclusions discussed in this thesis were not affected by this unknown ‘matrix effect’.

S3.2 Full day batch tests

In this section, the data for each full-day batch test is presented, with the measured TET concentrations and monitored environmental conditions as relevant.

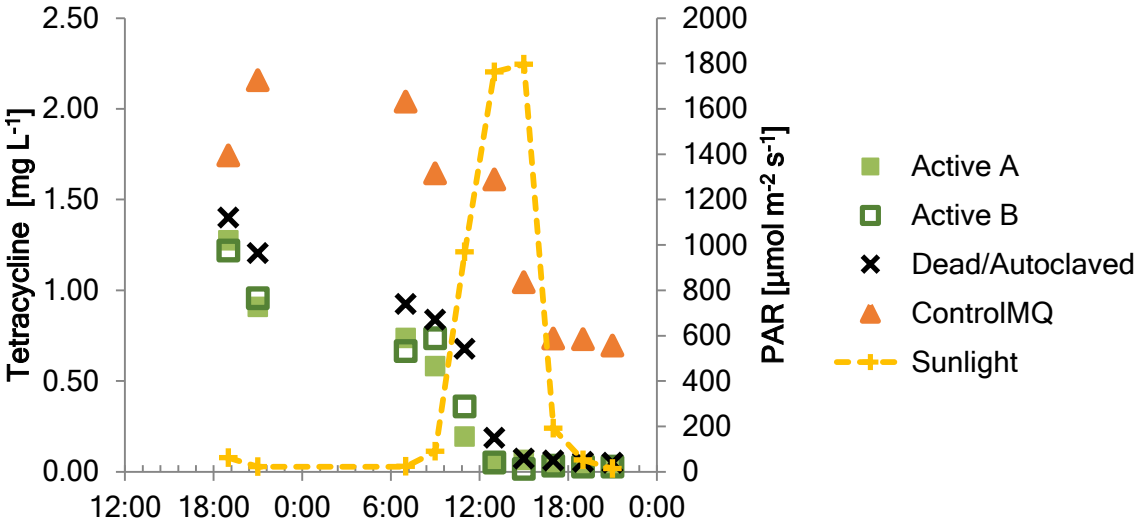

Figure 14: Tetracycline concentrations during full-day batch test 28th-29th May 2015

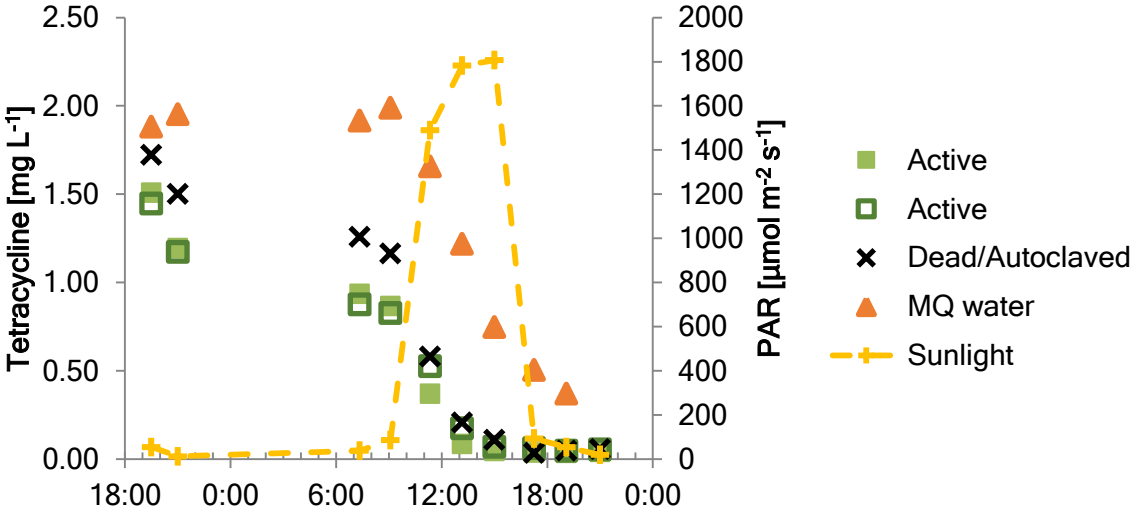

Figure 15: Tetracycline concentrations during full-day batch test 1st-2nd Jun 2015

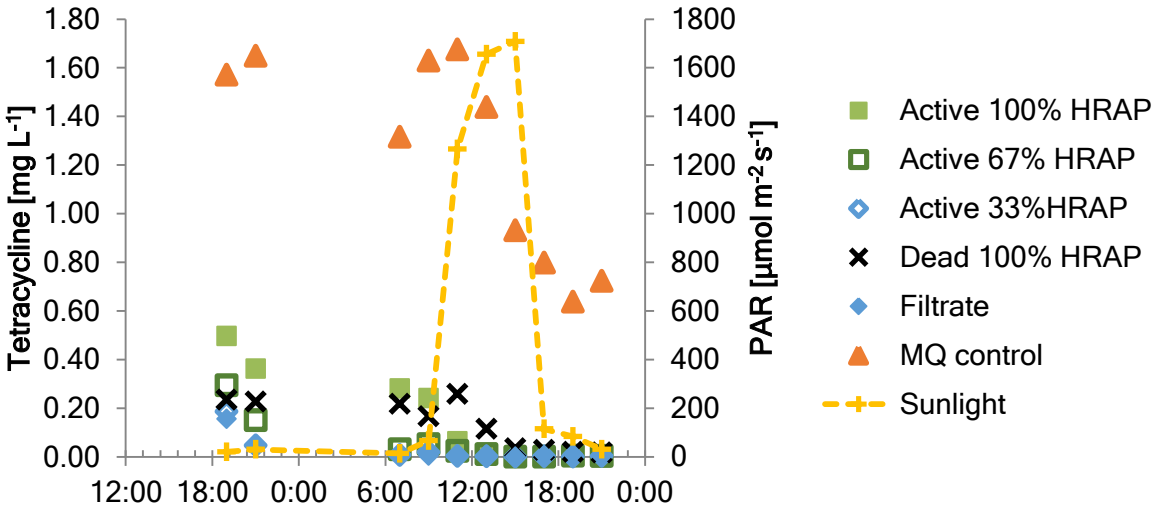

Figure 16: Tetracycline concentrations during full-day batch test 9th-10th Jul 2015

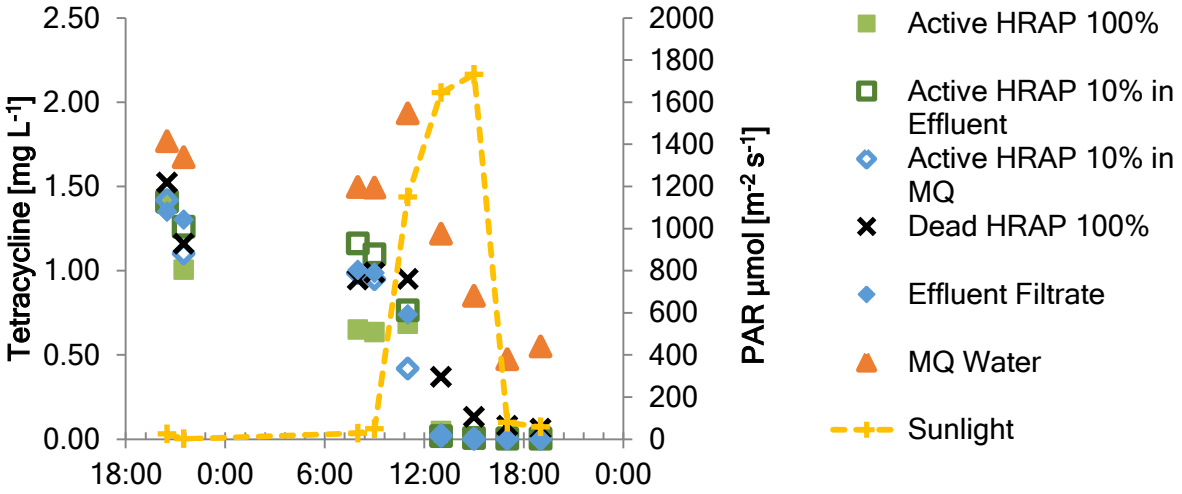

Figure 17: Tetracycline concentrations for the full day batch test 5th-6th Aug

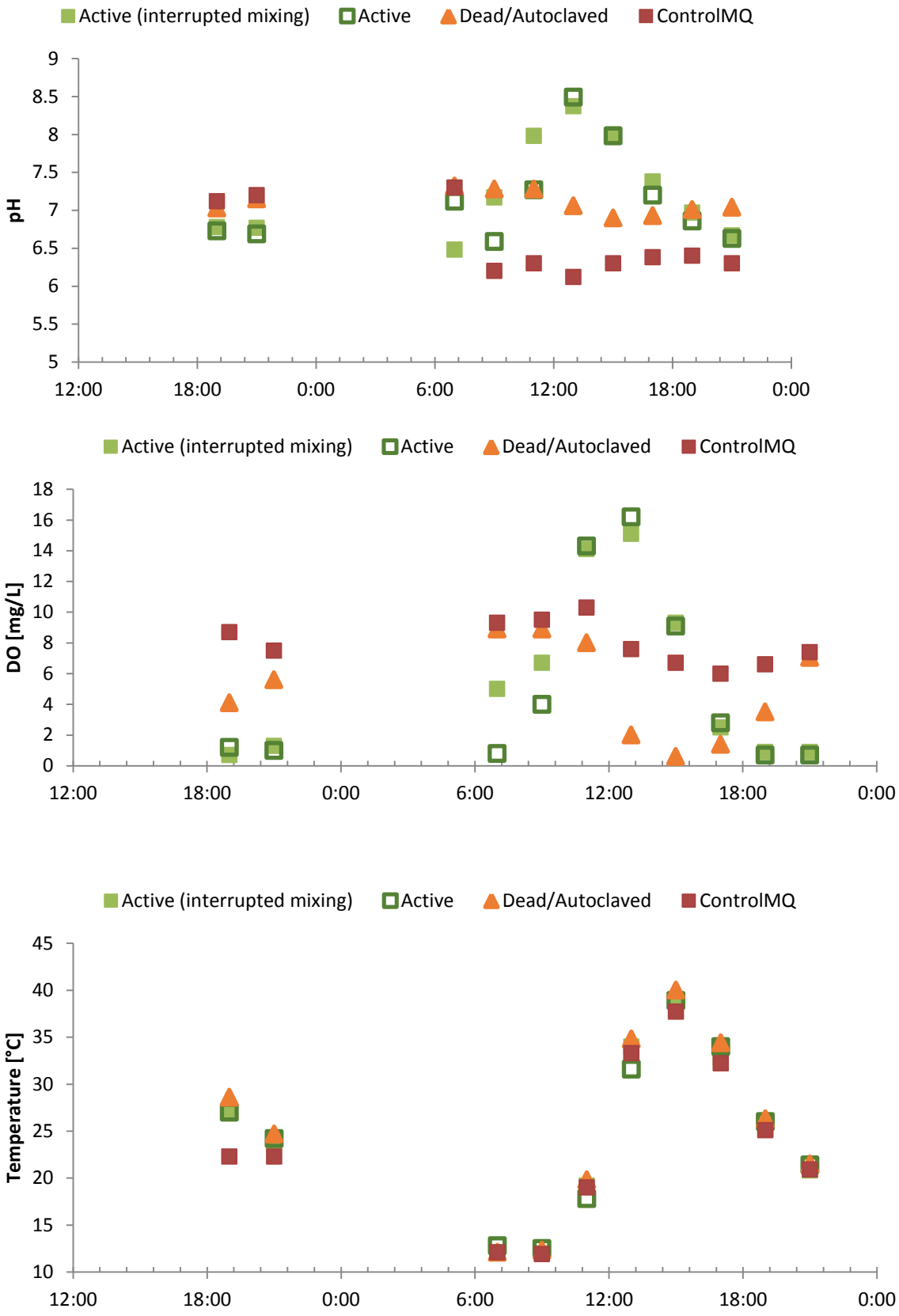

Figure 18: Environmental monitoring of the pH, DO, and temperature on 28th-29th May 2015

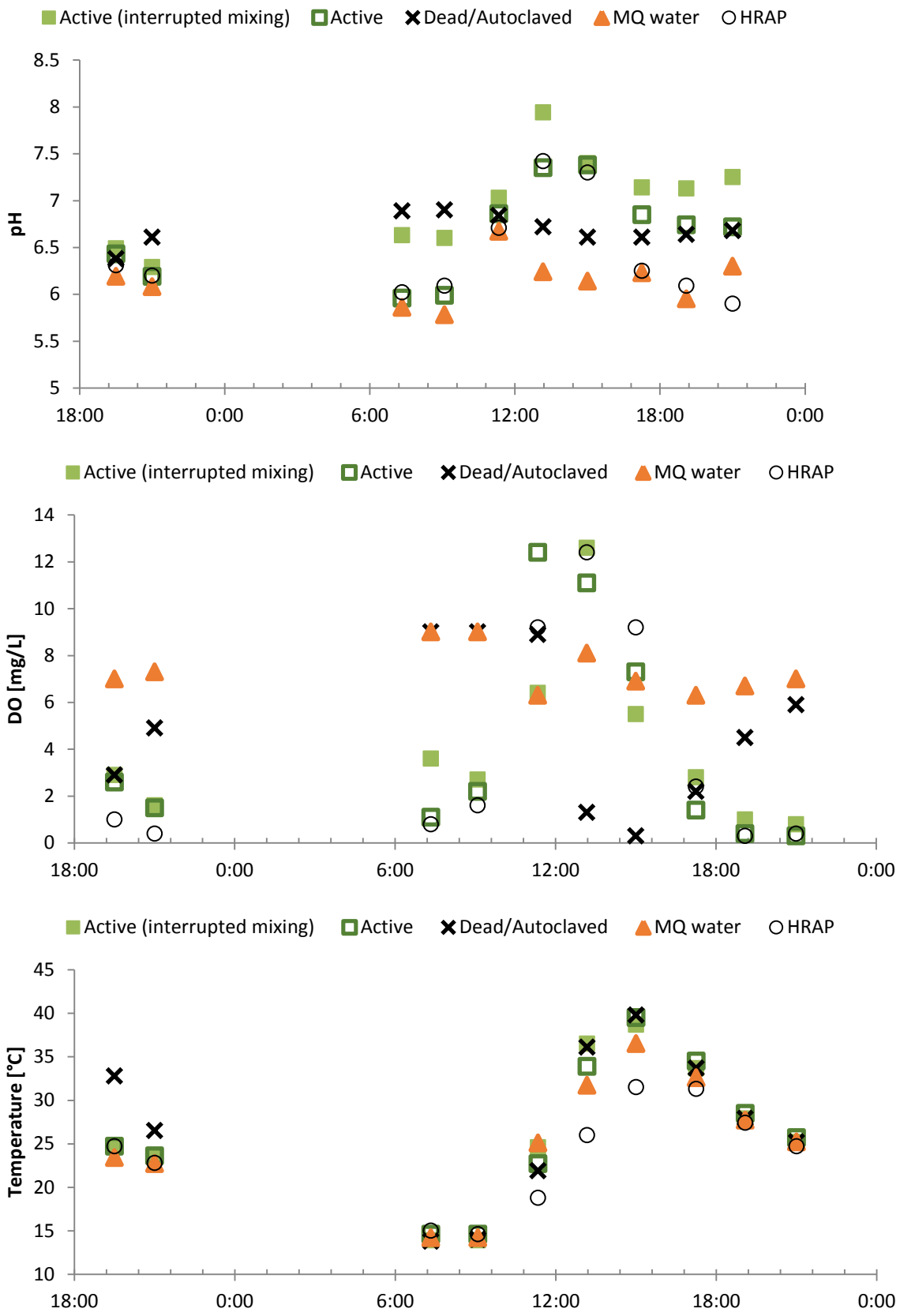

Figure 19: Environmental monitoring of the pH, DO, and temperature on 1st-2nd Jun 2015

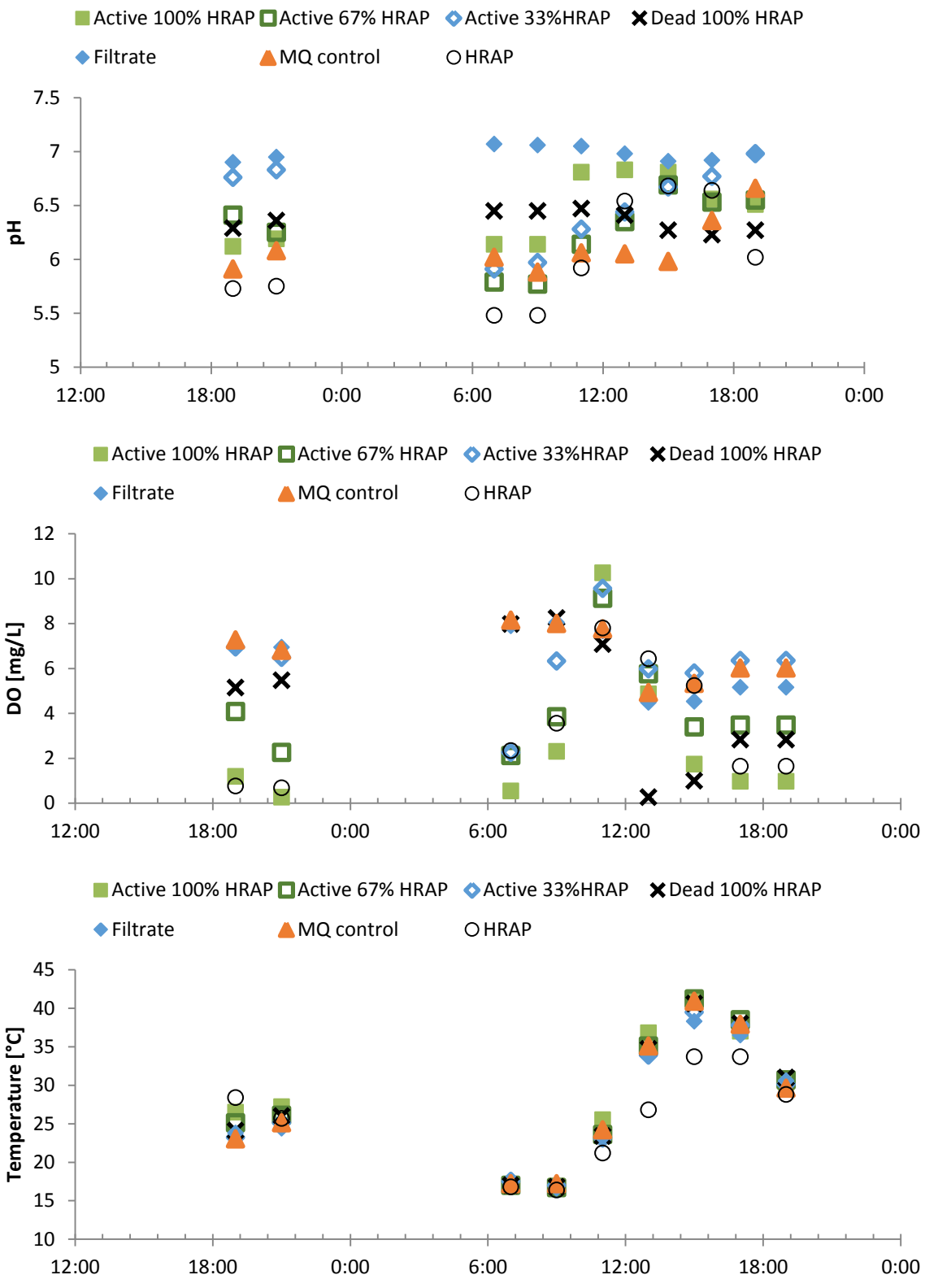

Figure 20: Environmental monitoring of the pH, DO, and temperature on 9th-10th Jul 2015

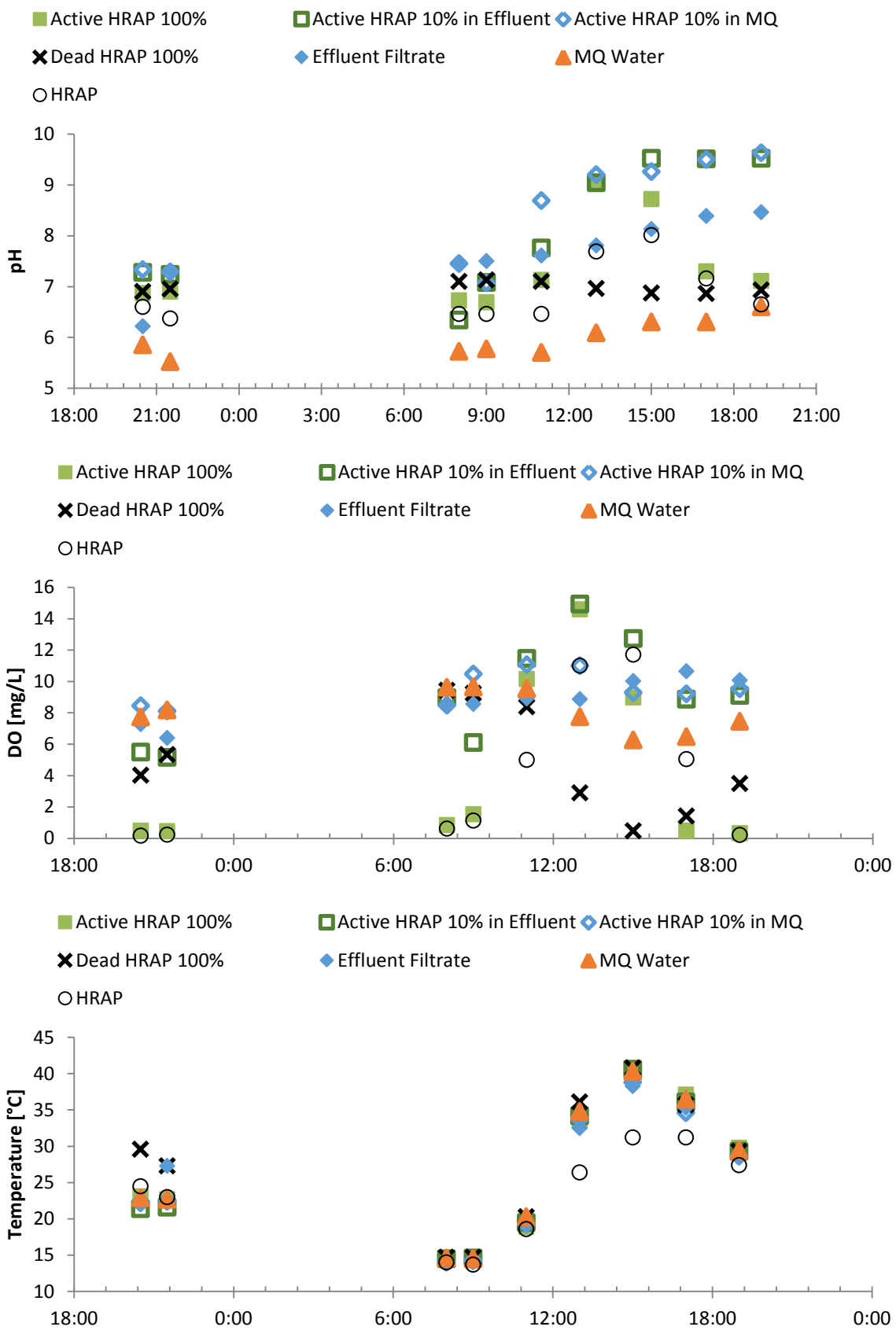

Figure 21: Environmental monitoring of the pH, DO, and temperature on 5th-6th Aug 2015

S3.3 pH adjustment test 4th Aug 2015 (4 h indoor, 1 h outdoor, TET only)

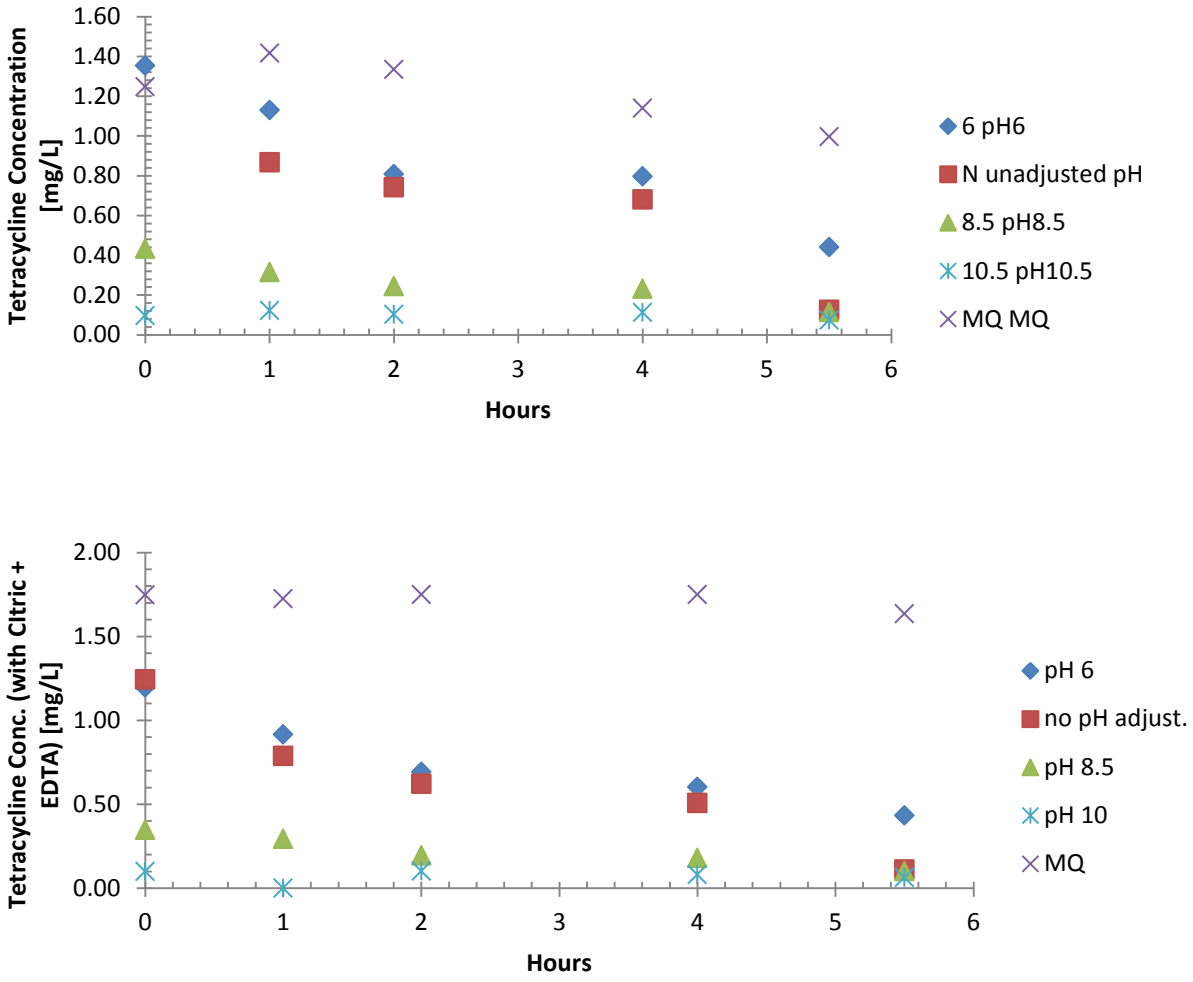

Figure 22: Tetracycline concentrations with and without citric acid + EDTA pretreatment (see Section S3.1) to HPLC samples for the pH adjustment batch test on 4th Aug 2015. PAR light intensity during the hour in light was ~ 718 µmol/m^2/s

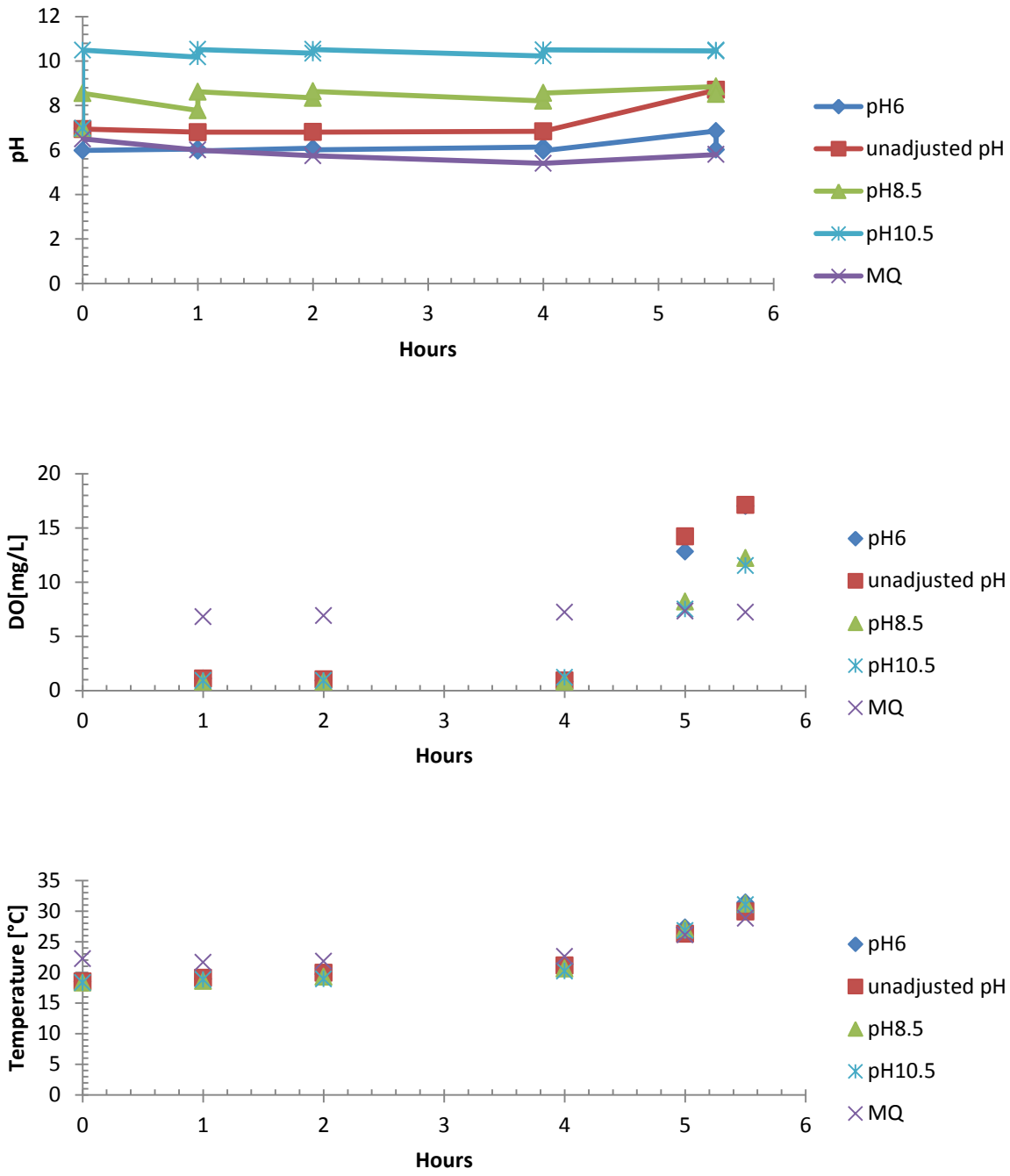

Figure 23: Environmental monitoring of the pH, DO, and temperature on 4 Aug 2015

Table 28: Volume of reagents added to adjust the pH on 4 Aug 2015

Time [h]	0	1	2	4	5.5	Total
pH6 [mL]	9	0.5	1	2	3.5	16
pH8.5[mL]	9	5.5	2	2	4	22.5
pH10.5 [mL]	55	10	5	9	0.5	79.5

S3.4 pH adjustment test in the light, 30th July 2015

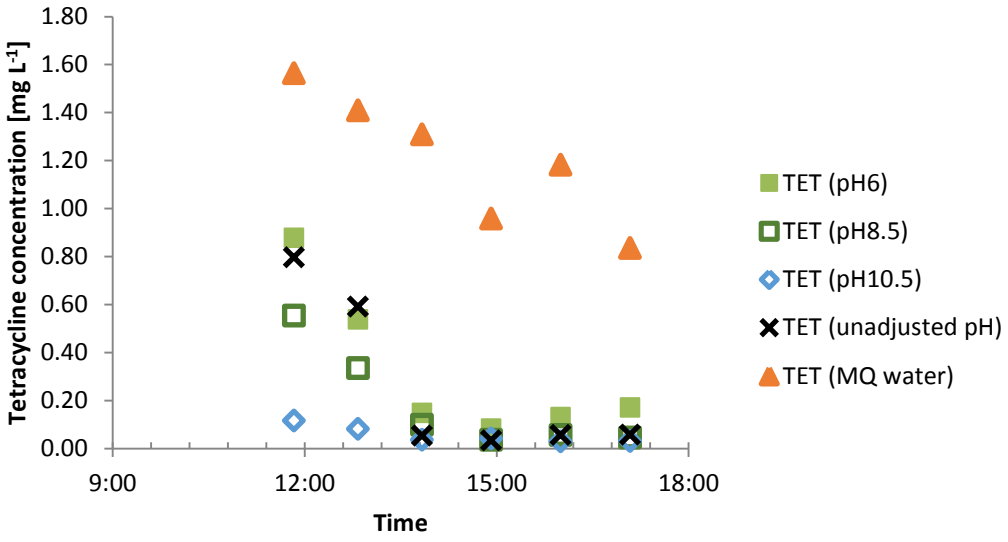

Figure 24: Tetracycline concentrations recorded for the batch test performed 30th July 2015 with pH manually adjusted before each sampling to the values shown in the legend using dropwise addition of 0.1 M NaOH or HCl.

S3.5 Sorption batch experiments

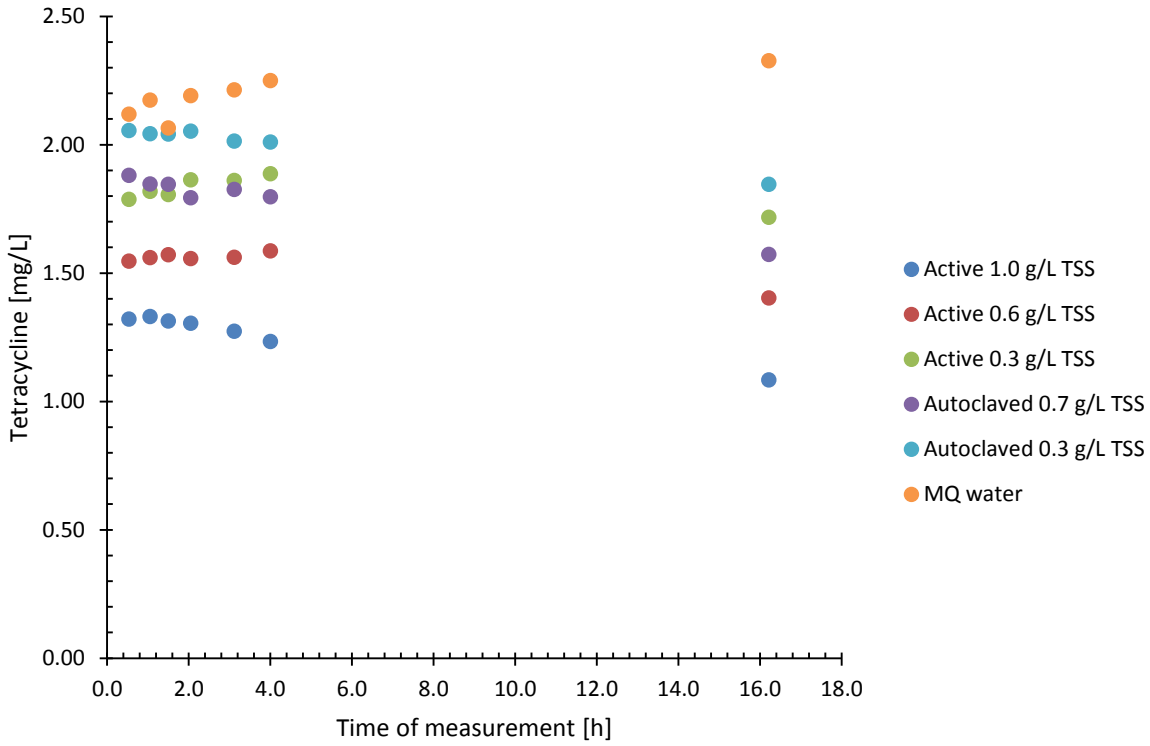

Figure 25: Tetracycline monitoring data for the sorption test 22nd June 2015

Table 29: Environmental parameters during the sorption test 22nd June 2015

	initial pH	pH at 4 h	pH at 8 hours	DO at 4 h [mg/L]	DO at 16 h [mg/L]	T [°C] at 4 h	T [°C] at 16 h
Active 1.0 g/L TSS	7.28	6.53	6.37	1.2	0.4	20.9	21.5
Active 0.6 g/L TSS	7.25	6.56	6.28	2.8	0.8	20.9	21.1
Active 0.3 g/L TSS	7.14	6.81	6.44	5.0	3.2	20.9	21.1
Autoclaved 0.7 g/L TSS	6.69	7.00	6.96	6.3	7.4	22	21.4
Autoclaved 0.3 g/L TSS	6.86	6.72	7.21	5.6	7.6	22	20.0
MQ water	6.4	8.01	5.8	7.5	7.9	21.8	19.9

Table 30: Numerical values of TET recorded during the sorption experiment 31st Aug 2015

	TET concentration [mg/L] without citric					TET concentration [mg/L] with citric				
Time (h)	**0**	**0.5**	**1**	**4**	**14**	**0**	**0.5**	**1**	**4**	**14**
0.2mg/L initial	0.17	0.13	0.11	0.09	0.08	0.20	0.16	0.15	0.13	0.10
0.5 mg/L initial	0.33	0.27	0.25	0.19	0.18	0.44	0.37	0.35	0.29	0.24
1 mg/L initial	0.72	0.61	0.56	0.44	0.38	0.92	0.79	0.63	0.73	0.54
2 mg/L initial	1.41	1.26	1.10	0.99	0.86	1.78	1.53	1.47	1.32	1.14
5 mg/L initial	4.09	3.41	3.28	2.81	2.62	4.37	4.05	3.87	3.55	3.34
10 mg/L initial	8.46	8.27	7.64	7.36	6.73	8.70	8.46	8.32	7.93	7.62

Table 31: Environmental parameters during the sorption test 31st Aug-1st Sep 2015

	initial pH	**pH at 4 h**	**pH at 8 hours**	**Initial DO [mg/L]**	**DO at 4 h [mg/L]**	**DO at 16 h [mg/L]**	**initial T [°C]**	**T at 4 h [°C]**	**T at 16 h [°C]**
0.2mg/L initial	6.47	6.46	6.50	0.2	0.1	0.17	23.6	21.4	21.2
0.5 mg/L initial	6.48	6.46	6.48	0.2	0.1	0.16	23.7	21.4	21.2
1 mg/L initial	6.45	6.45	6.46	0.2	0.1	0.19	23.7	21.5	21.3
2 mg/L initial	6.47	6.46	6.46	0.2	0.1	0.14	23.8	21.1	21.3
5 mg/L initial	6.47	6.46	6.45	0.2	0.1	0.15	23.7	20.9	20.1
10 mg/L initial	6.47	6.47	6.45	0.2	0.1	0.2	23.7	20.5	20.0

Table 32: Mass balance of sorbed and aqueous TET concentrations after 4 h incubation with algal-bacterial biomass in 2.5 L batch reactors under dark conditions

Initial Conc. of TET supplied	**Aqueous TET Conc. at 4 h**	**Sorbed TET Conc. at 4 h**	**Kd[a] at 4 h**	**Total TET recovered, sorbed + aqueous**	**Total TET recovered, sorbed + aqueous**
[mg L^{-1}]	**[mg L^{-1}]**	**[mg g_{TSS}^{-1}]**	**[L g_{TSS}^{-1}]**	**[mg]**	**[%]**
0.2	0.13	0.19	1.5	0.78	79
0.5	0.29	0.33	1.1	1.54	87
2	1.32	0.98	0.74	5.58	100
5	3.55	2.02	0.57	13.8	108
10	7.93	4.14	0.52	29.7	111

[a] Sorption partition coefficient $K_d = \frac{[mg_{sorbed.antibiotic}/g_{TSS}]}{[mg_{dissolved.antibiotic}/L_{solution}]}$

Table 33: Mass balance of sorbed and aqueous TET concentrations after 14 h incubation with algal-bacterial biomass in 2.5 L batch reactors under dark conditions

Initial Conc. of TET supplied	**Aqueous TET Conc. at 14 h**	**Sorbed TET Conc. at 14 h**	**Kd[a] at 14 h**	**Total TET recovered, sorbed + aqueous**	**Total TET recovered, sorbed + aqueous**
[mg L^{-1}]	**[mg L^{-1}]**	**[mg g_{TSS}^{-1}]**	**[L g_{TSS}^{-1}]**	**[mg]**	**[%]**
0.2	0.10	0.23	2.2	0.80	79
0.5	0.24	0.39	1.6	1.54	87
1	0.54	0.67	1.2	2.89	96
2	1.14	1.15	1.0	5.53	100
5	3.34	2.37	0.71	14.1	108
10	7.62	3.83	0.50	28.2	113

S4. Appendix Four – Quality control tests

In this section, a few of the quality control experiments performed are summarised. The four sections discussed below, are: The confirmation of average light intensity for the lab HRAPs and indoor beaker batch experiments by actinometry; the monitoring of TET influent degradation; confirmation that full recovery of TET is obtained with 2 mL eluent MeOH during the solid phase extraction procedure (SPE), and recovery tests for TET using different filters to prepare the samples for the HPLC.

S4.1 Actinometry (25.4.14)

The method for chemical actinometry using potassium ferric oxalate (PFO) developed by Hatchard and Parker(1956) [a] and adapted by Quentin Bechet was used. Due to light exposure, the PFO reduces to iron(II) ions, which can be detected by absorbance using phenanthroline as an complexing agent. This method is explained thoroughly for the HRAP, with the same procedure, but with smaller volume and different sampling times, used to measure the light intensity for the indoor batch 100 mL beakers.

In summary: 0.006 M PFO in 0.1 N H_2SO_4 was prepared and 7 L was added to each HRAP; 0.5 mL samples were taken at times 0, 5, 10, 15, 20, 25, 30, 40, 60 min; samples were immediately added to a measuring cylinder with 9.5 mL 0.1 N H_2SO_4, 5 mL buffer (0.6 N sodium acetate with 0.36 N H_2SO_4), and 2 mL phenanthroline monohydrate (2.72 g/L); the solution was made up to 25 mL with RO water, mixed and transferred to a cuvette; at the end of the experiment (minimum 30 min time sitting in the cuvette), absorbance readings were taken at 510 nm (peak wavelength for the phenanthroline/iron(II) complex).

[a] Hatchard, C.G., Parker, C. a., 1956. A New Sensitive Chemical Actinometer. II. Potassium Ferrioxalate as a Standard Chemical Actinometer. Proc. R. Soc. A Math. Phys. Eng. Sci. 235, 518–536. doi:10.1098/rspa.1956.0102

A calibration curve for iron(II) concentration was prepared, using ferrous sulfate solution prepared from a freshly prepared stock solution at 0.0003 M in 0.1 N H_2SO_4. For each calibration point, X mL of $FeSO_4$ solution [X=0, 0.5, 2, 4, 6, 8, 10 mL], 10-X mL 0.1 N H_2SO_4, 5 mL buffer, and 2 mL phenanthroline were added to a measuring cylinder, the volume made up to 25 mL with RO water, and the absorbance read after at least 30 min.

For monochromatic light, the light intensity received (I_0, W/m^2) is related to the rate of production of Fe^{2+} (β, mol/s) by the following equation:

$$I_0 = \frac{\beta}{q} N_A \frac{hc}{\lambda} \frac{1}{A}$$

Where q is the quantum efficiency (1.21 (Hatchard and Parker 1956)), N_A is Avogrado's number ($6.02 * 10^{23}$ mol^{-1}), h is Plank's constant ($6.6 * 10^{-34}$ J.s), c is the speed of light in a vacuum ($3 * 10^8$ m s^{-1}), λ is the wavelength of the light, and A is the surface area (m^2). β is calculated from the slope $[Fe^{2+}]$/time (s), multiplied by the total volume (7 L).

Since our light is not monochromatic, but a spectrum, the equation must be integrated over the spectrum, and multiplying λ by the fraction (f(λ)) of the light energy emitted by the light bulb in each interval [λ to λ+dλ] and the percent absorbance of the PFO solution in that interval (1- T_λ) where T_λ is the transmittance of the PFO at wavelength λ. So the equation becomes:

$$I_0 = \frac{\beta}{q} N_A \frac{1}{A} \frac{hc}{\int_\lambda \lambda * f(\lambda) * (1 - T_\lambda)}$$

For these calculations a 5 nm step was chosen and the equation was manually integrated, using the points taken from the graphs in Figure 26 shown below.

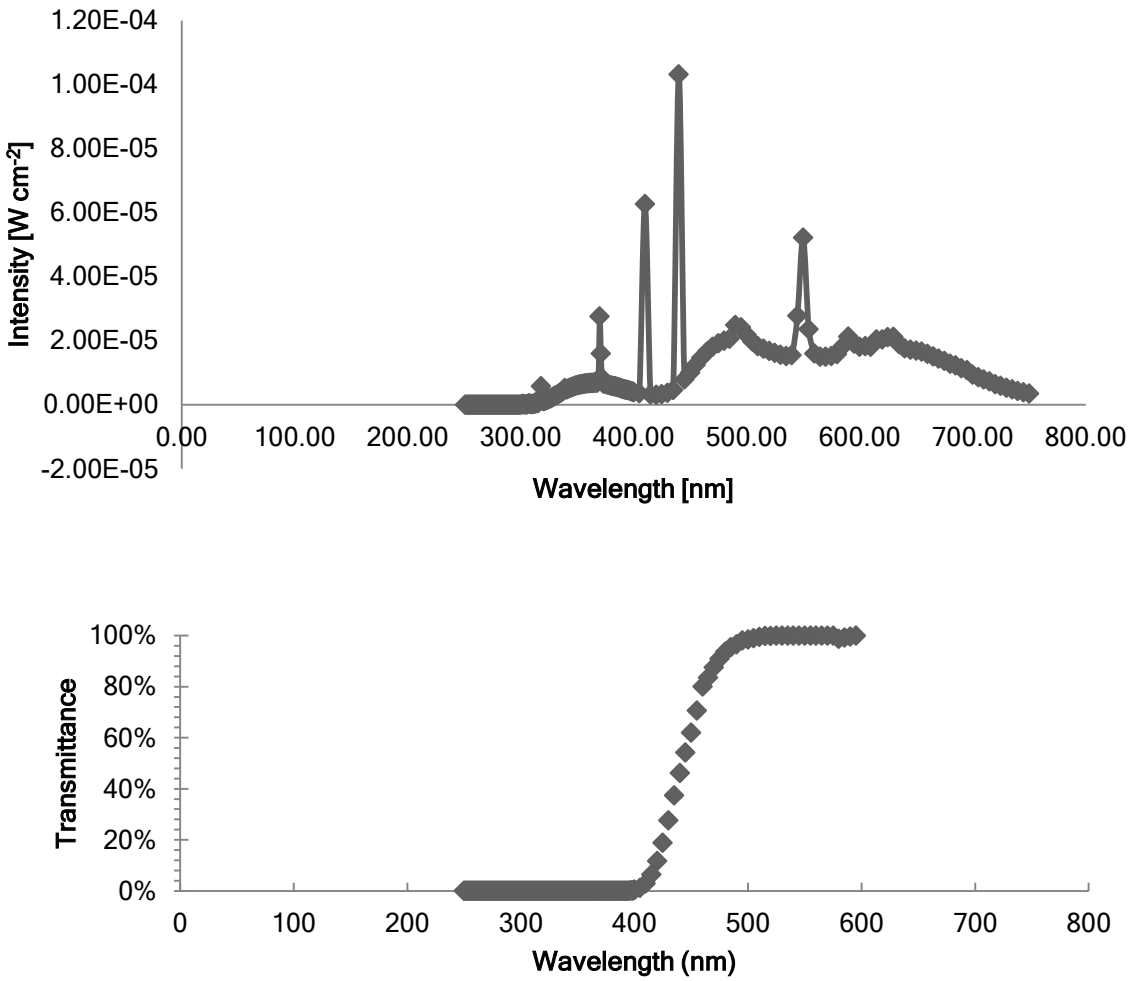

Figure 26: Relative intensity spectra of T8 fluorescent full spectrum Viva-Lite® tubes used above the HRAPs (Optronics OL756 spectroradiometer (Orlando, FL, USA)), and transmittance of the PFO solution measured by spectrophotometer in quartz cuvettes.

Only the lights above the pond were on while the experiment was being conducted. All other artificial light in the room was turned off, thought there was some ambient light from the windows. The fluorescent lights were fully ‘warmed up’, switched on for 3 hours before the experiment began.

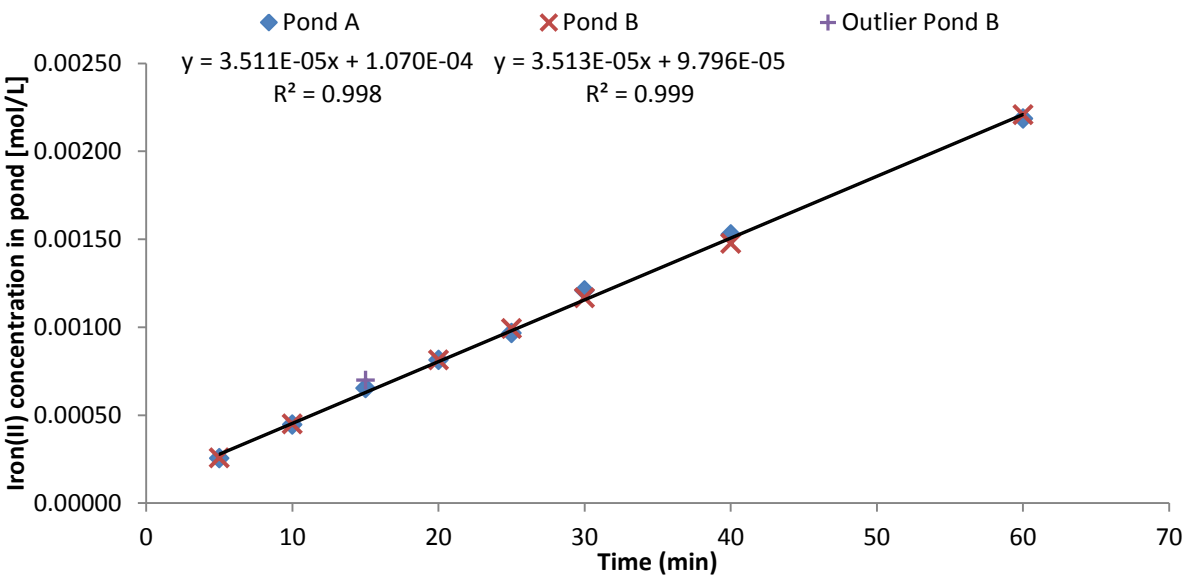

Figure 27: Accumulation of Iron(II) in the HRAPs over time.

The measured concentrations of Iron(II) over time provided a strong linear slope seen in

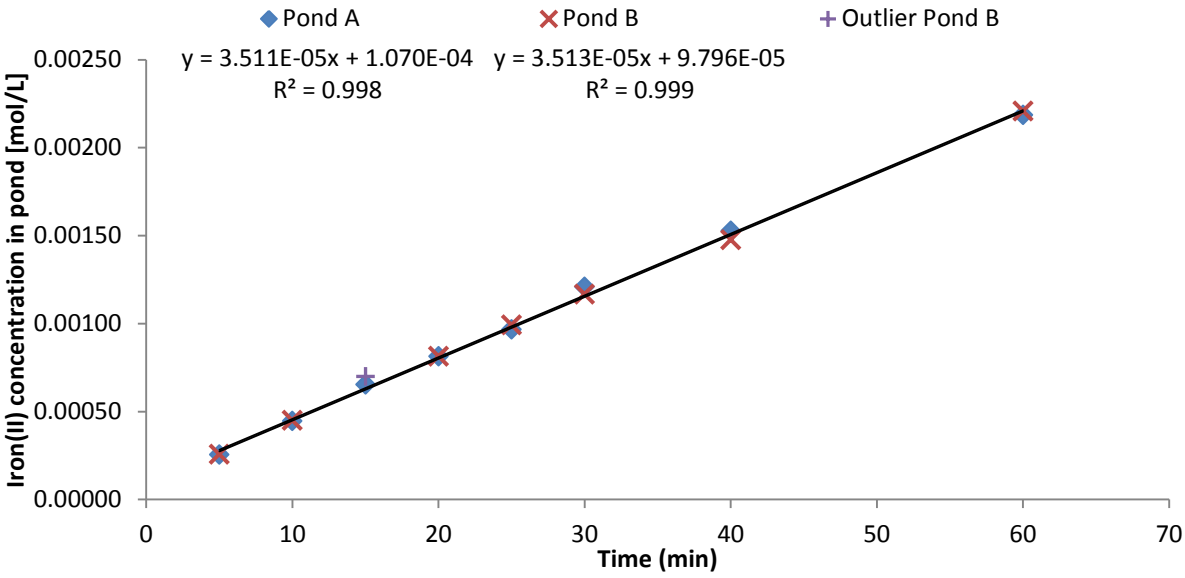

Figure 27, as expected, with more than 99.9% agreement between the slopes, indicating that the two ponds received equal irradiation from the lights above.

The rate of production of iron(II) in the ponds was converted to total irradiance received. This was 46 W/m^2 for both Pond A and Pond B. There was a 2% standard

error on the slope, but errors from the assumed constant quantum efficiency may increase error up to 5-10% (estimate).

This procedure was repeated for the 100 mL beakers used in the batch experiments, with a mean 15.9 ± 2.0 (s.d.) W/m^2 calculated for the beakers at the experimental light intensity.

S4.2 Tetracycline influent monitoring for lab-scale HRAPs

The tetracycline in the wastewater influent was monitored both for the lab HRAP and for the pilot HRAPs. However, due to sorption to the WW solids and inconsistent recorded values for the monitored influent concentrations, all calculations in this thesis were based on the theoretical concentration of influent tetracycline using the concentration and volume of the stock solution added to the WW influent. The variability of the influent concentrations is seen in Figure 28 for the lab HRAPs.

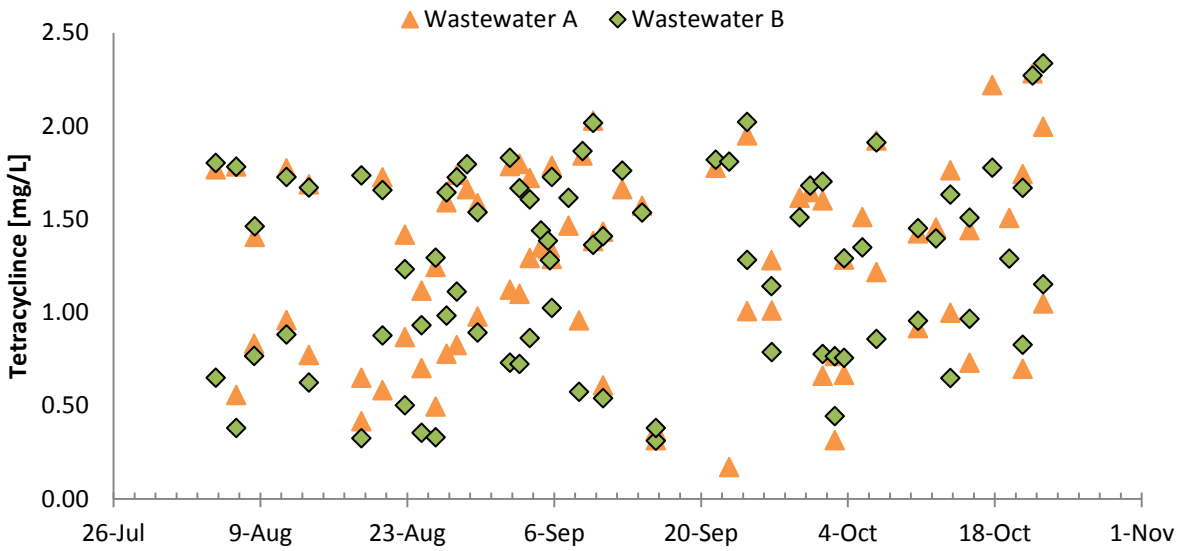

Figure 28: Wastewater influent monitoring for the lab HRAPs (A & B), recorded with both freshly prepared influent WW, and concentrations after 1-2 days in the feed tanks. Theoretical concentration of TET was 2 mg/L.

Sorption by itself (in closed containers) was not enough to explain the drop in aqueous TET concentrations in the influent feed tanks (Figure 29).

It was hypothesized that there may have been some degradation in the influent tanks due to the highly aerobic nature (around >80% dissolved oxygen) that came from keeping the 5 L tanks mixed. To remove this potential degradation before reaching the HRAP from the equation, before the final two periods the tetracycline stock solution in purified water was pumped separately to the wastewater. This did not change the flow-

rates, as purified water was previously added to compensate for evaporation in the lab-scale HRAP. There was a slight but insignificant decrease in the tetracycline concentration when this change to the influent pumping regime was made, although as noted in the thesis, this may have caused a change in the sorption partition coefficient with the biomass (much higher sorption partition coefficients measured after the change). If degradation in the WW influent tanks was previously significant, then we would expect an increase in HRAP TET concentrations. Therefore we concluded that despite the aqueous concentration of tetracycline decreasing in the influent tanks, this was not significant to the conclusions made, and calculations should be based on the 2 mg/L influent concentration expected from the added tetracycline. It is also possible that the matrix effects observed and discussed in Section 4.4.1 in the thesis played a role.

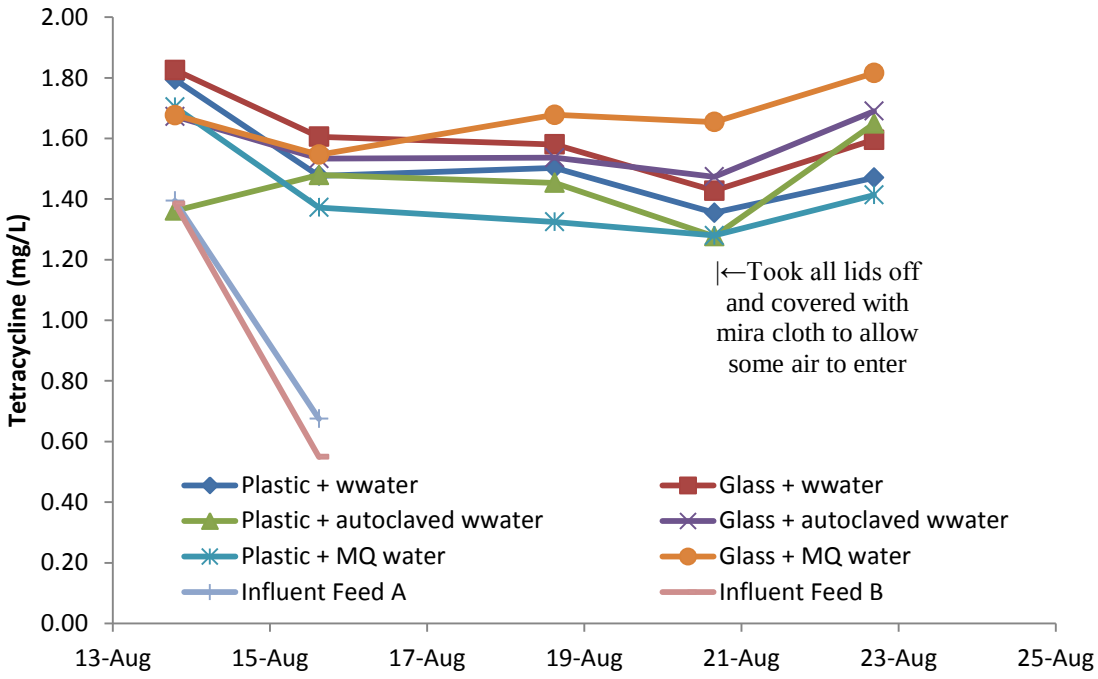

Figure 29: Tetracycline stability (intended concentration 2 mg/L) in closed glass or plastic

S4.3 SPE recovery tests

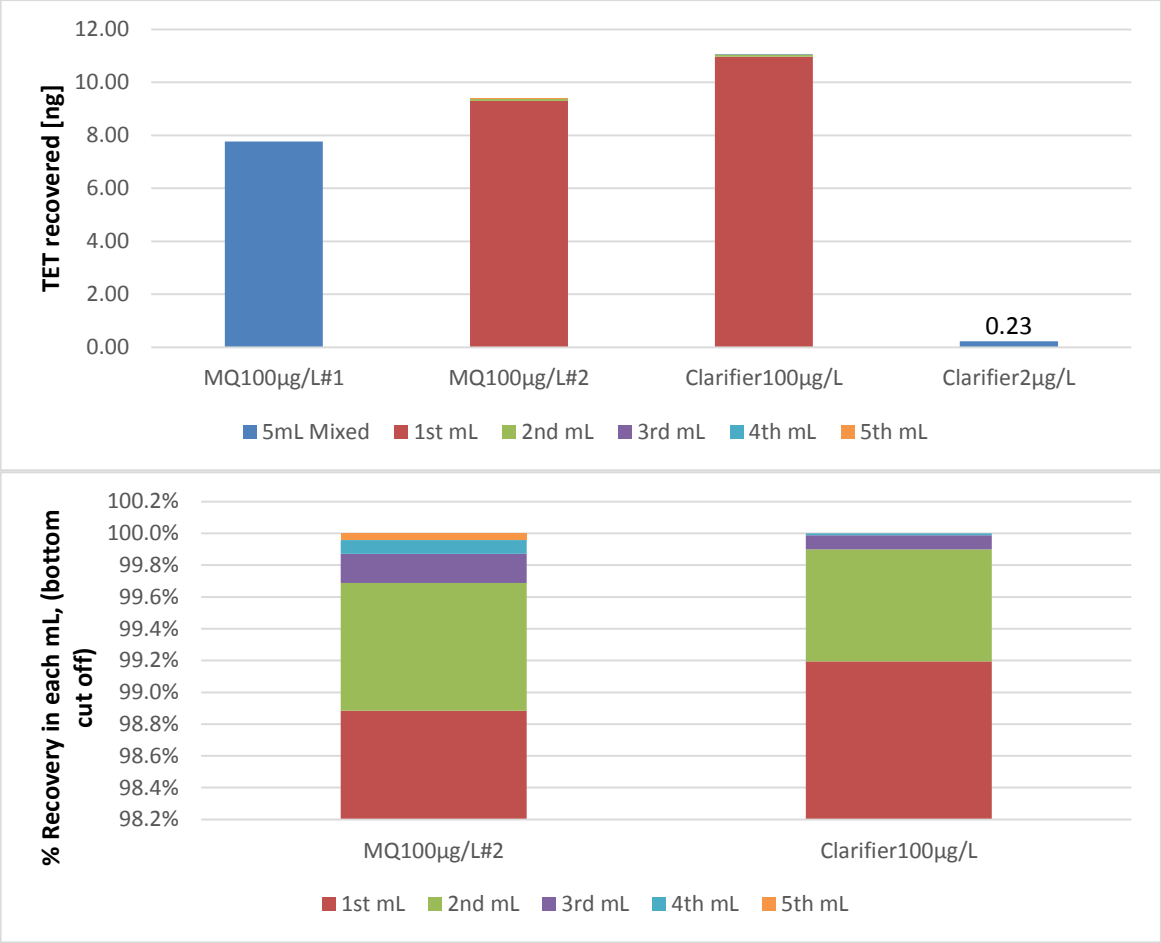

Figure 30: SPE recovery tests showing that essentially all the tetracycline was recovered in the first 2 mL of MeOH eluent, and better results were obtained with 2 mL eluents than 5 mL of MeOH eluent. 100 µg/L samples should report 10 ng TET recovered, and the 2 µg/l sample should report 0.2 ng TET recovered

S4.4 Recovery tests for TET filtration

A test was done to confirm which filtration/purification method was best to remove particles prior to the HPLC analysis of TET. It was discovered (Figure 31) that syringe filters were the best in this case, and the GFC filters had the curious problem that biomass in the sample significantly decreased recovery of TET after filtration, but these filters were acceptable for phosphate buffers and MQ water.

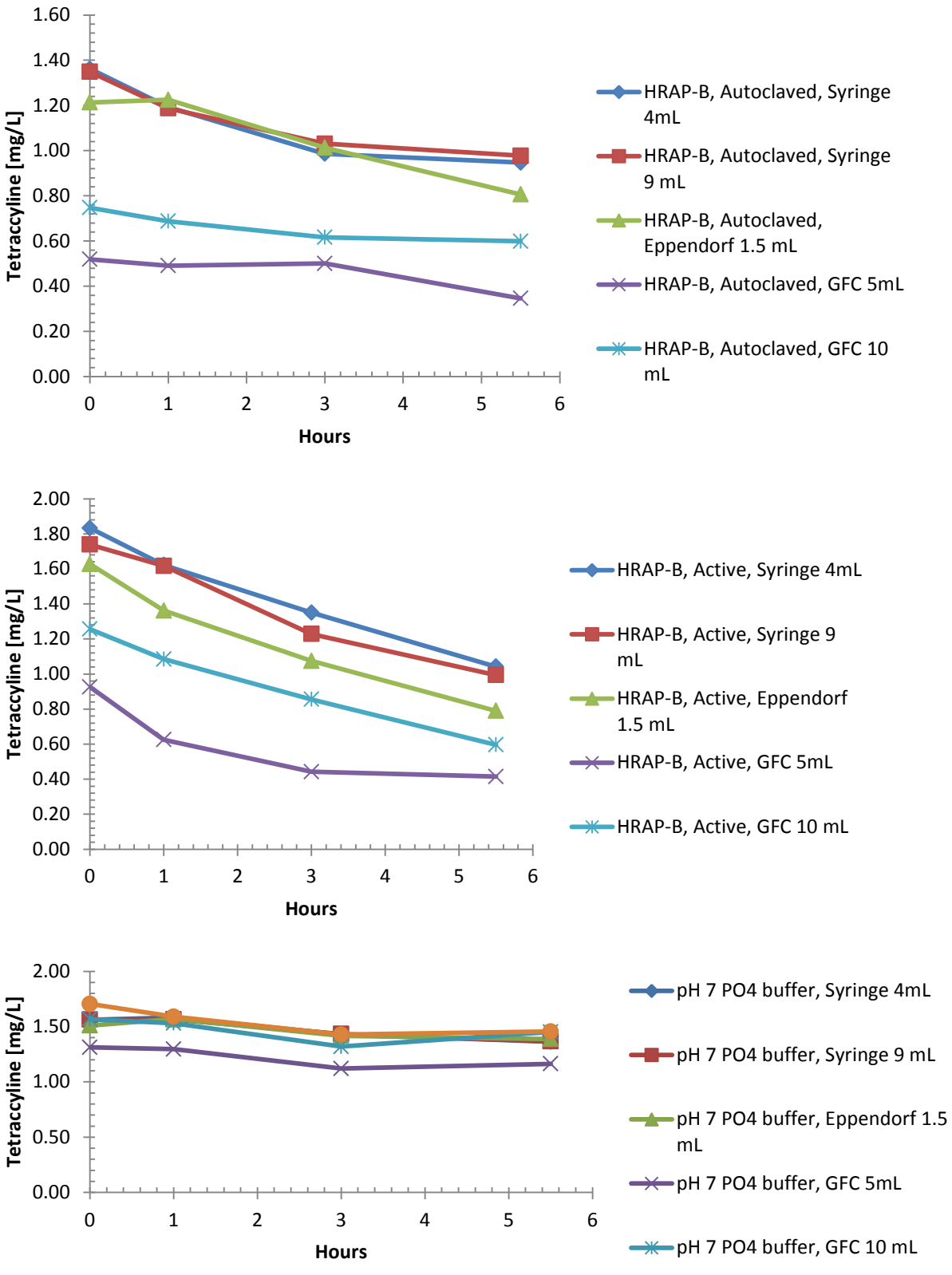

Figure 31: Recovery tests for TET through GFC glass fibre 47 mm filters, Millipore 0.2 µm cellulose acetate syringe filters, or centrifugation in Eppendorf tubes.

S5. Appendix Five – Matlab model predictions and fitting

S5.1 Matlab fitting of the kinetic model parameters to the experimental data from the batch tests

Table 34: Summary of the k_{1L} values and K_d values fitted to the full-day outdoor batch experimental data

Date	Biomass conditions	k_{1L}	R^2 [a]	K_d	k_{1s}	k_{1ds}
28-May	Active 100% HRAP biomass	4.20E-05	0.96	0.8	15	14.4
28-May	Autoclaved 100% HRAP biomass	1.70E-05	0.96	0.8	15	14.4
1-Jun	Active 100% HRAP biomass	2.10E-05	0.97	0.85	15	13.6
1-Jun	Autoclaved 100% HRAP biomass	1.50E-05	0.99	0.4	15	28.8
5-Aug	Active 100% HRAP biomass	6.00E-05	0.99[b]	1.4	15	8.93
5-Aug	Autoclaved 100% HRAP biomass	1.40E-05	0.91	0.8	15	15.6

[a] R^2 values were calculated after excluding the first data point, due to the initial rapid sorption skewing the R^2 results.
[b] One outlier at 11am was excluded

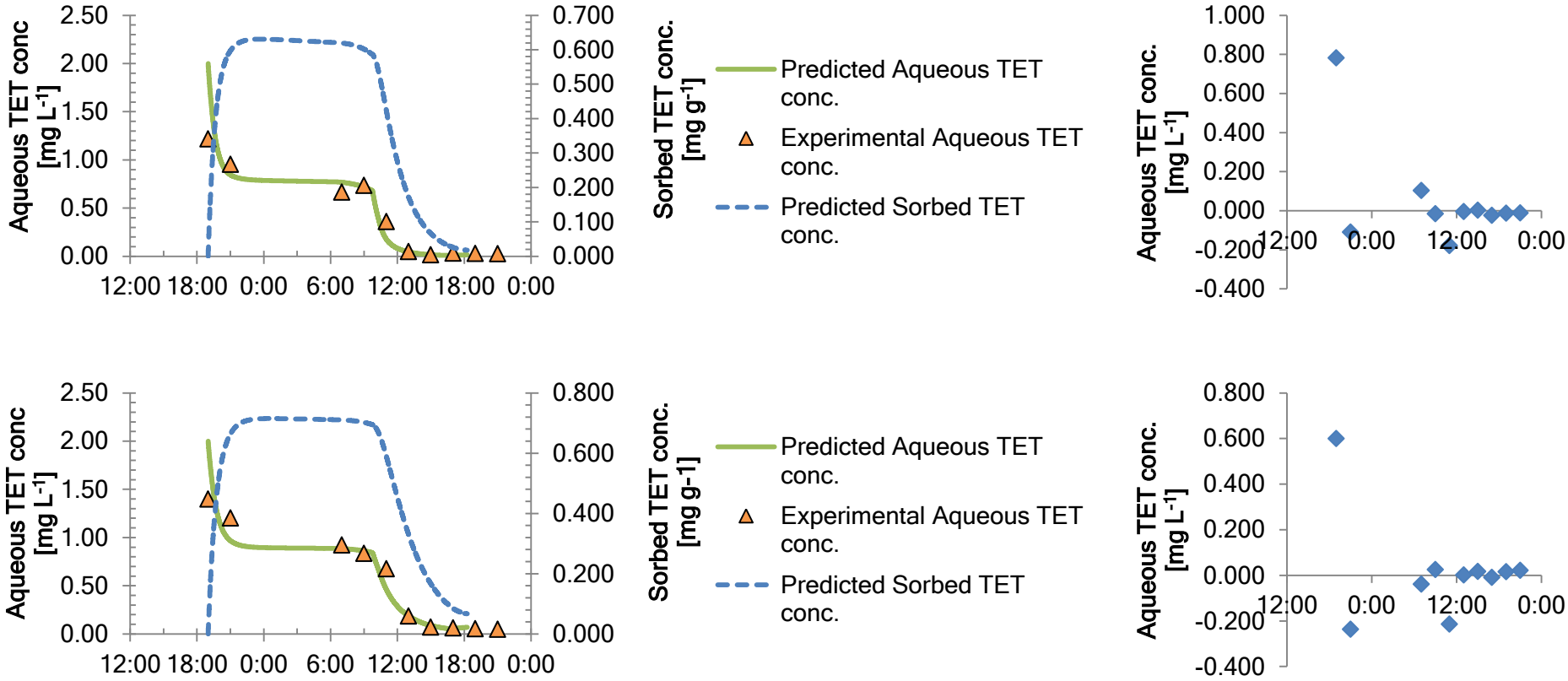

Figure 32: Predicted aqueous and sorbed TET concentrations for the batch test 28th May 2015 after fitting using Matlab. Top: active biomass, Bottom: autoclaved biomass. Residuals for aqueous TET concentrations are shown on the right.

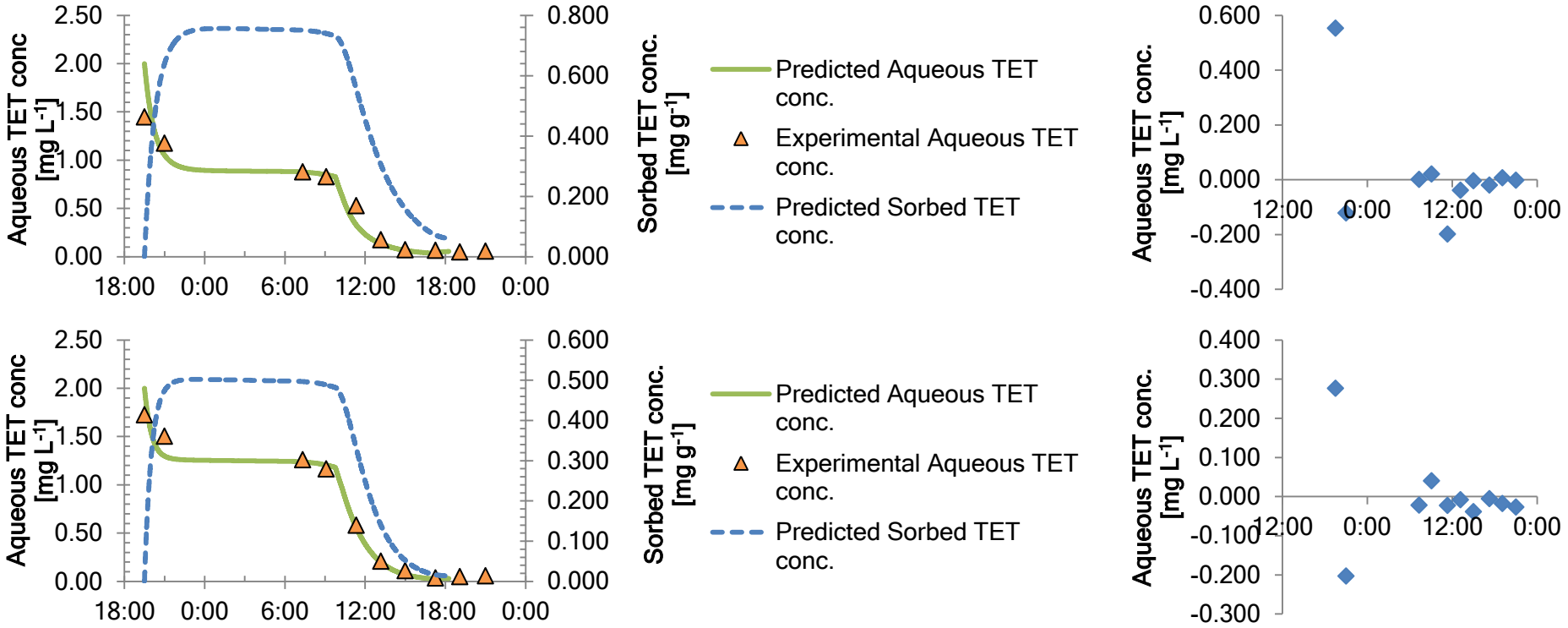

Figure 33: Predicted aqueous and sorbed TET concentrations for the batch test 1st June 2015 after fitting using Matlab. Top: active biomass, Bottom: autoclaved biomass. Residuals for aqueous TET concentrations are shown on the right.

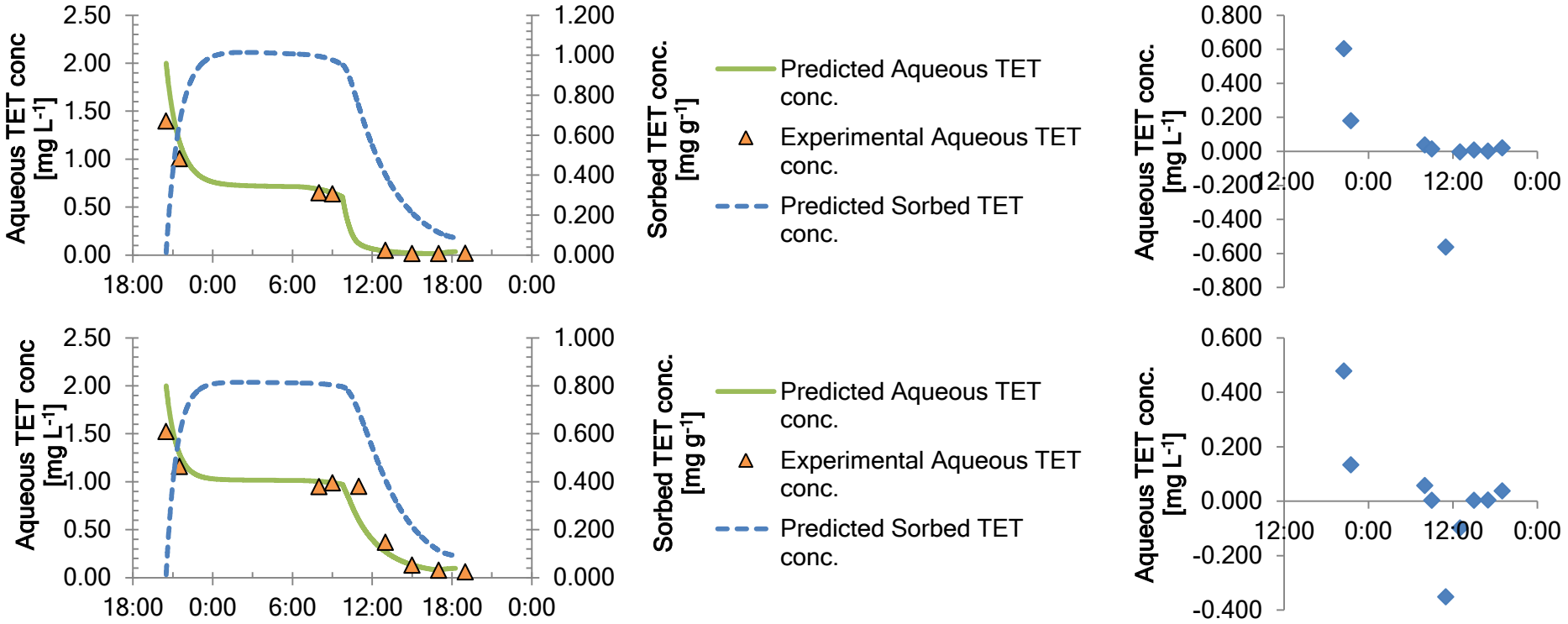

Figure 34: Predicted aqueous and sorbed TET concentrations for the batch test 5th Aug 2015 after fitting using Matlab. Top: active biomass, Bottom: autoclaved biomass. Residuals for aqueous TET concentrations are shown on the right.

S5.2 Matlab modelled predictions of TET removal in pilot HRAP pulse tests

Table 35: Constants used for predicting TET concentrations during the pulse tests in the pilot HRAP

Constants	Average value	High error bound	Low error bound
k_{1L} [L µmol^{-1}]	1.2 x10^{-5}	1.7 x10^{-5}	0.8 x10^{-5}
K_d [L g^{-1}]	3.0	4.5	1.4

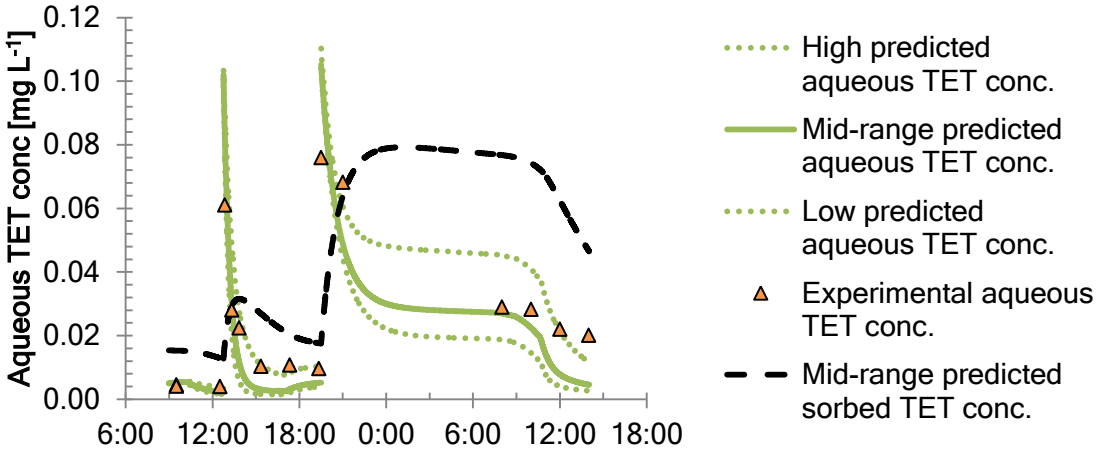

Figure 35:Predictions for the TET pulse test 7th Aug 2015

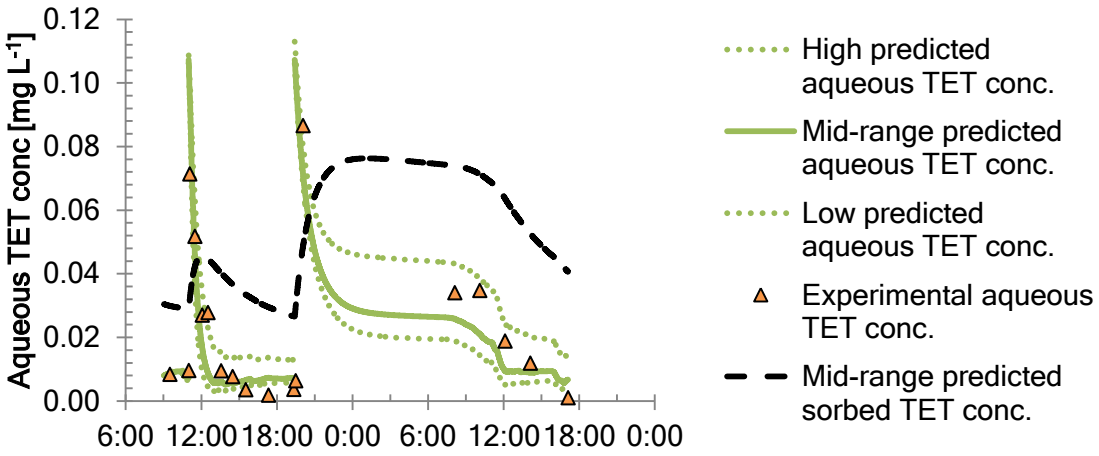

Figure 36:Predictions for the TET pulse test 13th Aug 2015

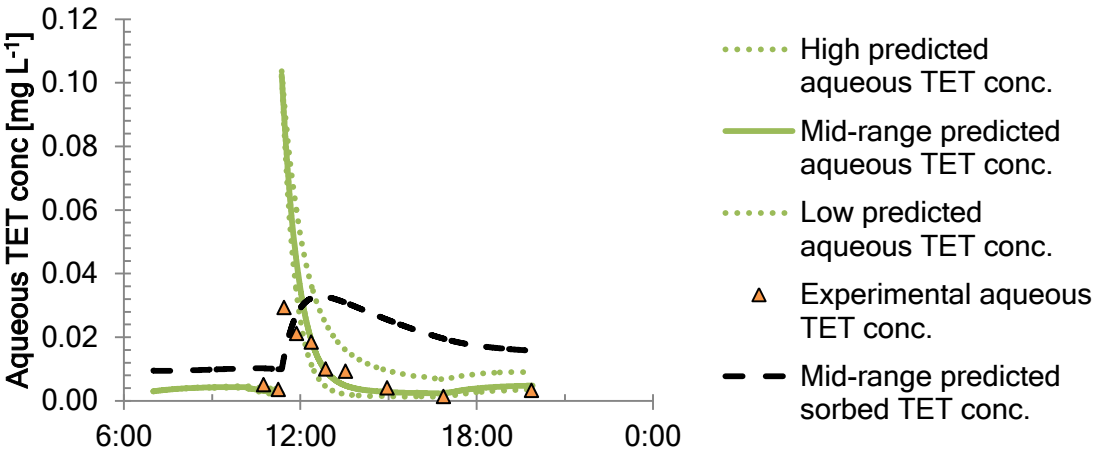

Figure 37:Predictions for the TET pulse test 18th Aug 2015

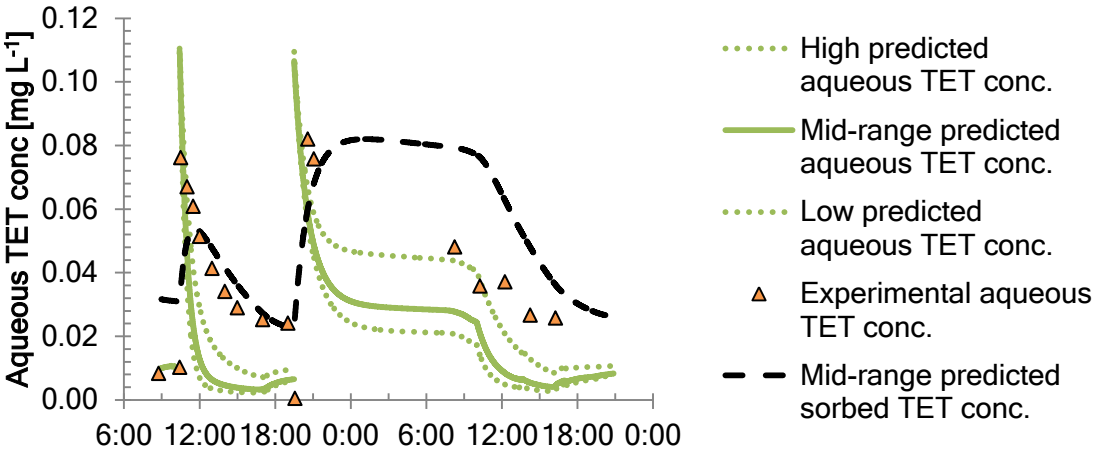

Figure 38:Predictions for the TET pulse test 20th Aug 2015

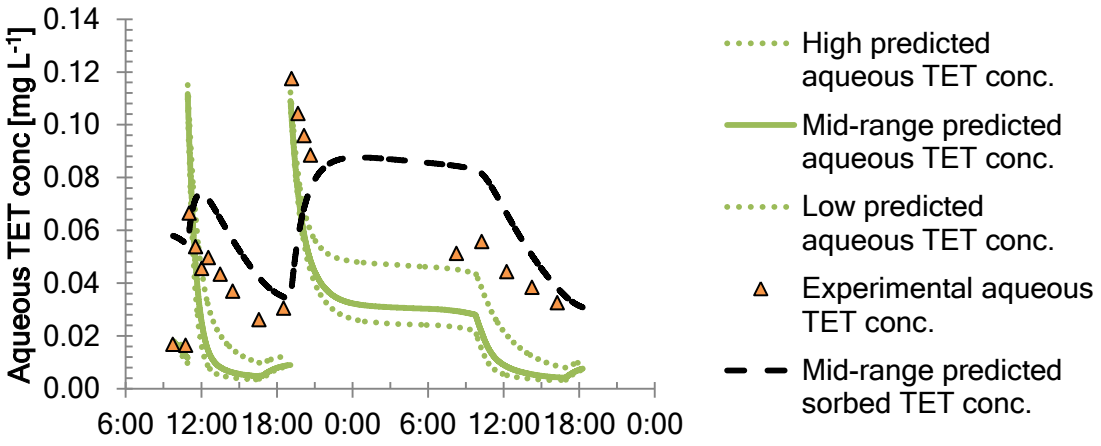

Figure 39:Predictions for the TET pulse test 27th Aug 2015

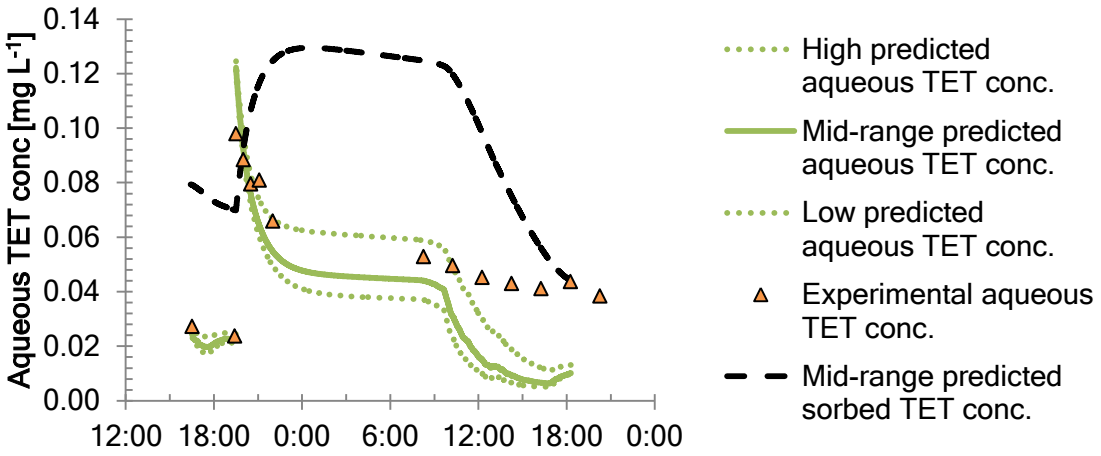

Figure 40:Predictions for the TET pulse test 31st Aug 2015

S5.3 Matlab fitting of the model to experimental data from pulse tests

Table 36: Summary of the fitted first order rate constants using Matlab simulations, the graphs of which are presented in the following subsections. The kinetic sorption constant k1s was held constant at 15 d^{-1}.

	k1L 'Day 1'	**k1L 'Day 2'**	**Kd**	**k1ds**	**R^2**
	L µmol^{-1}	L µmol^{-1}	L g^{-1}	d^{-1}	-
7-Aug-15	1.8E-05	7.0E-06	3.4	4.0	0.76
13-Aug-15	3.0E-05	3.5E-05	1.7	6.5	0.88
18-Aug-15	4.0E-05		3.0	4.4	0.84
20-Aug-15	3.0E-06	9.0E-06	3.0	4.4	0.94
27-Aug-15	7.0E-06	6.0E-06	1.8	7.3	0.88
31-Aug-15		3.0E-06	2.6	5.5	0.94
20-Mar-15		2.5E-05	1.8	13.9	0.92
25-Mar-15		2.7E-05	3.0	8.3	0.72

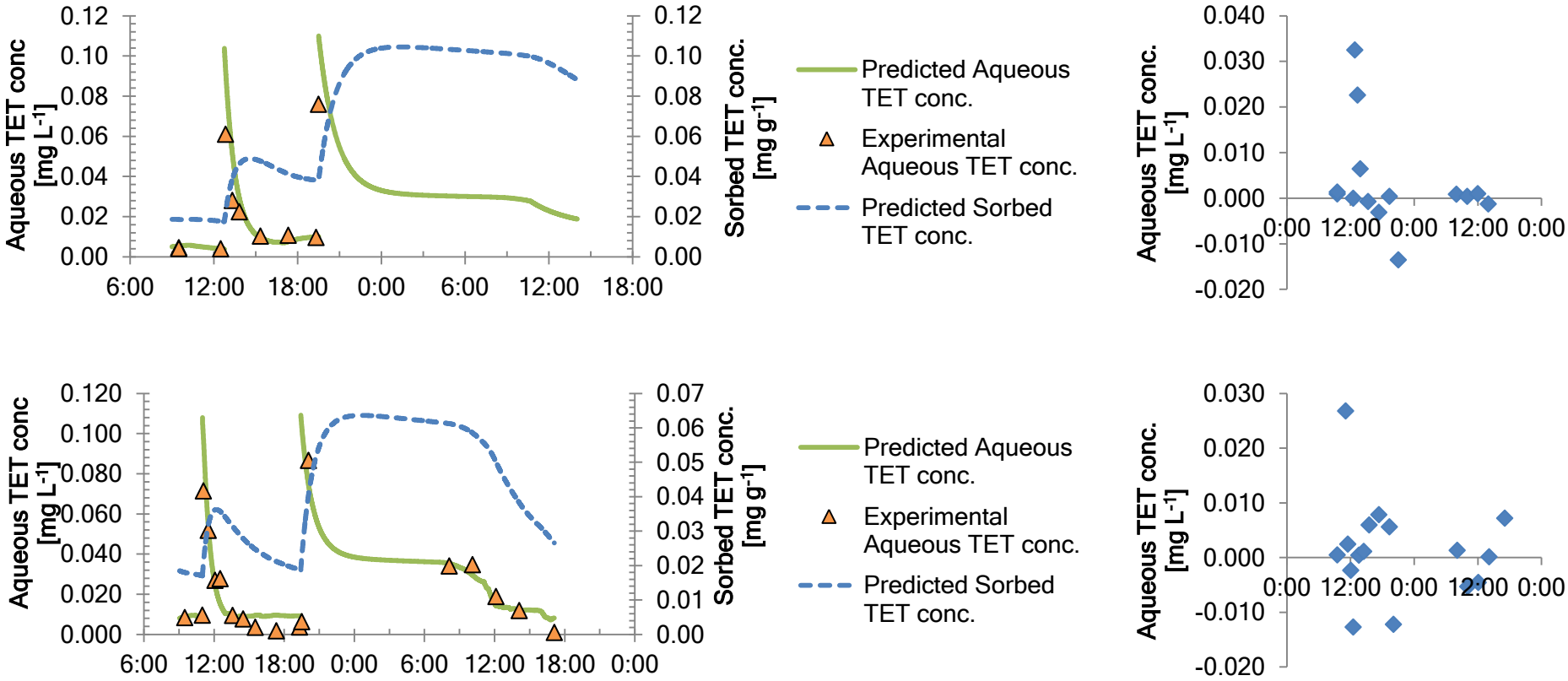

Figure 41: Predicted aqueous and sorbed TET concentrations for the pulse tests in the 180 L pilot HRAP after fitting using Matlab.

Top: 7th-8th Aug 2015, Bottom: 13th-14th Aug 2015. Residuals for aqueous TET concentrations are shown on the right.

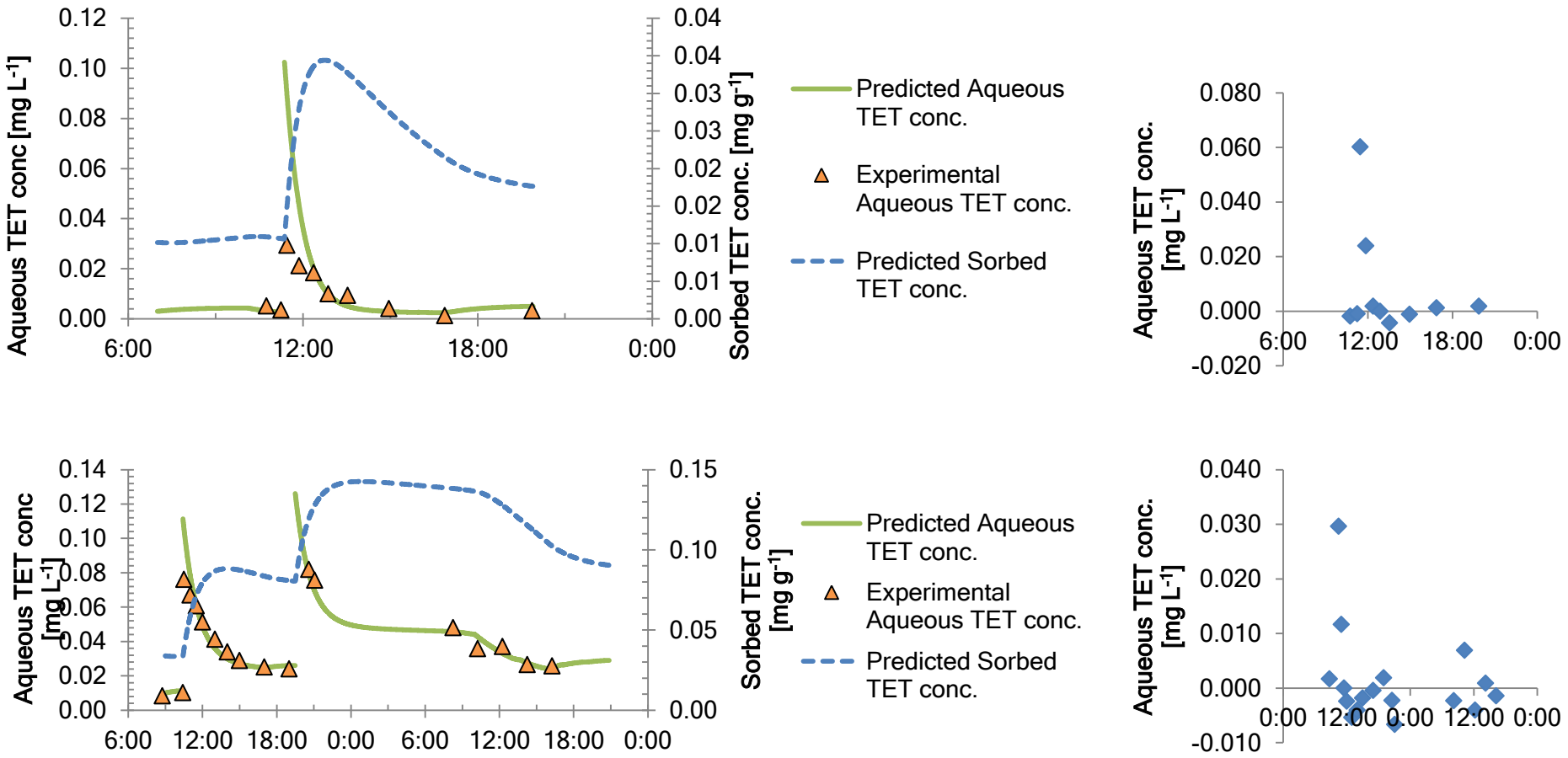

Figure 42: Predicted aqueous and sorbed TET concentrations for the pulse tests in the 180 L pilot HRAP after fitting using Matlab.

Top: 18th Aug 2015, Bottom: 20th-21st Aug 2015. Residuals for aqueous TET concentrations are shown on the right.

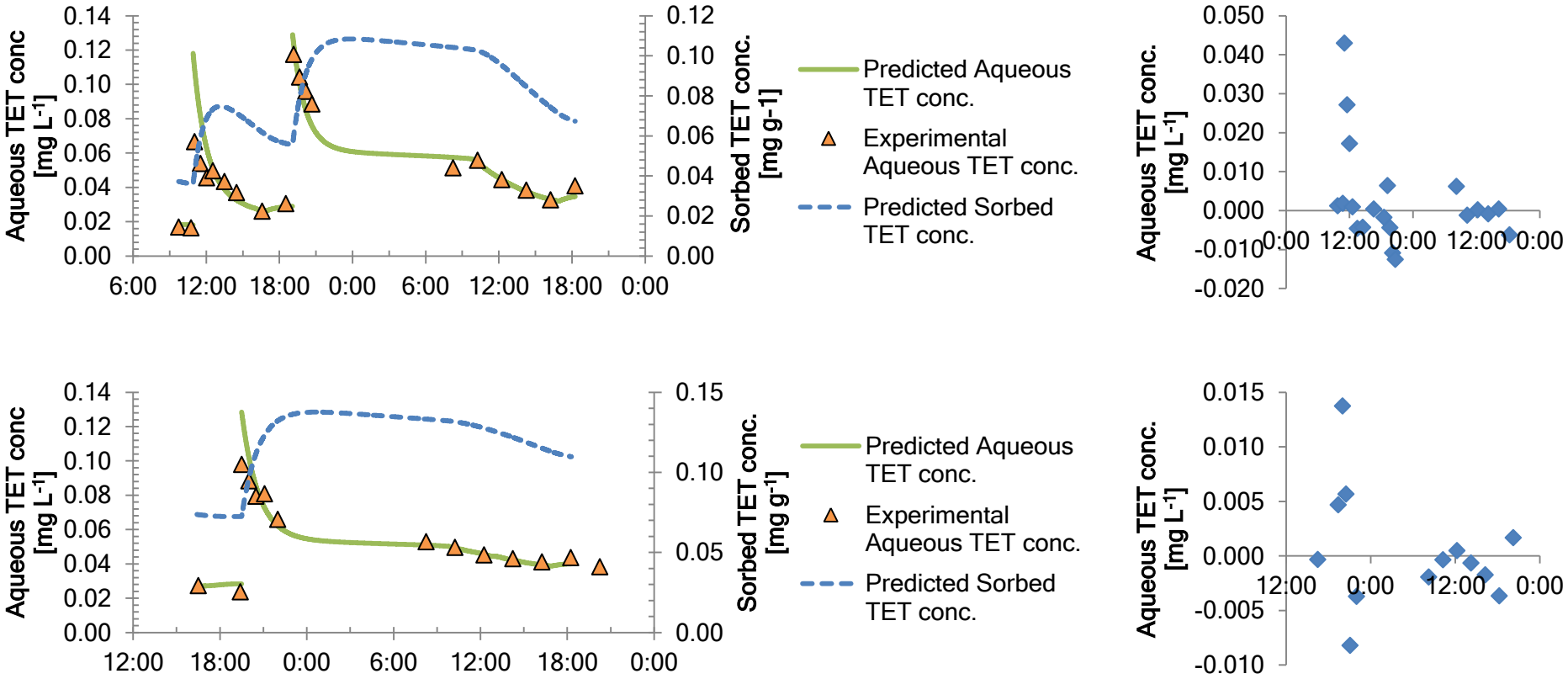

Figure 43: Predicted aqueous and sorbed TET concentrations for the pulse tests in the 180 L pilot HRAP after fitting using Matlab.

Top: 27th -28th Aug 2015, Bottom: 31st Aug-1st Sep 2015. Residuals for aqueous TET concentrations are shown on the right.

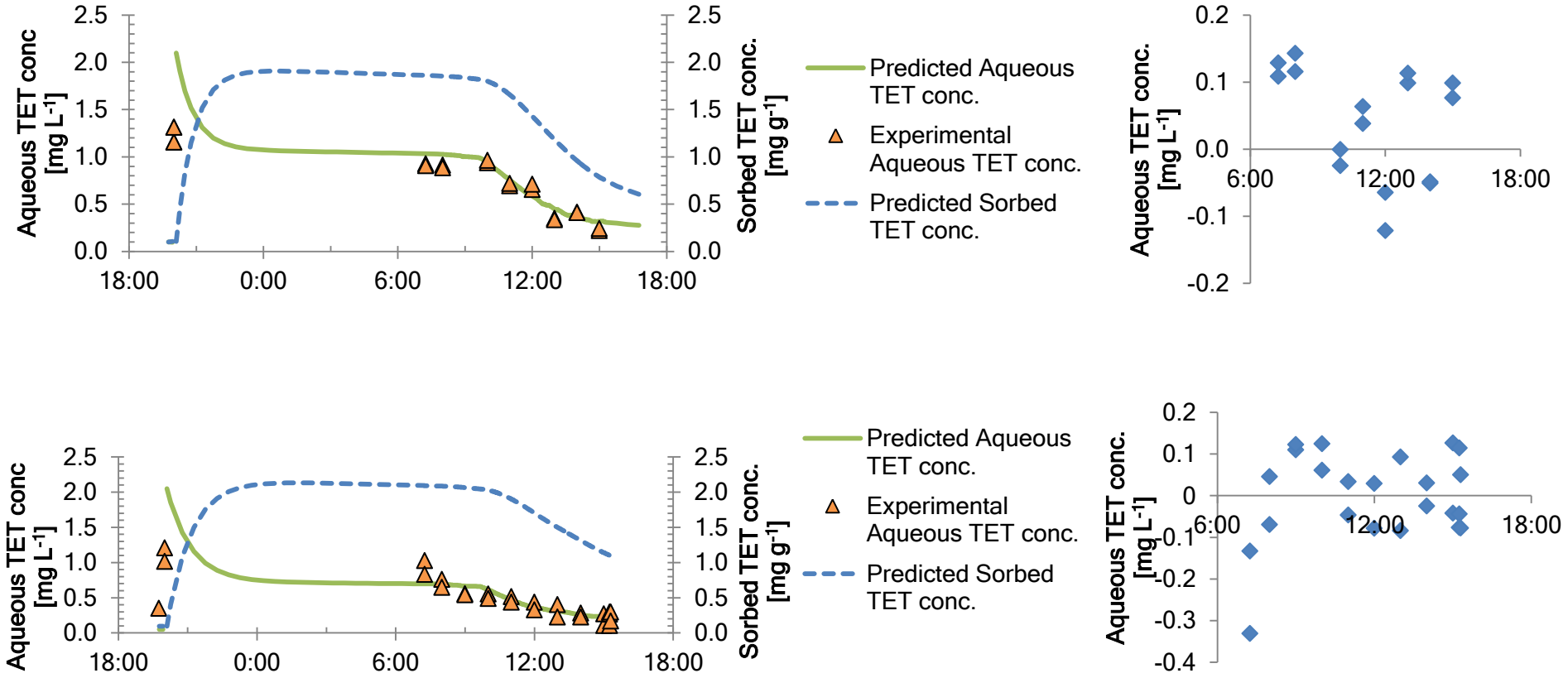

Figure 44: Predicted aqueous and sorbed TET concentrations for the pulse tests in the 900 L pilot HRAP after fitting using Matlab.

Top: 19th-20th March 2015, Bottom: 24th-25th March 2015. Residuals for aqueous TET concentrations are shown on the right.

S5.4 Sensitivity analysis for TET removal in algal ponds with continuous TET influent, based on Aug light levels

In this section, a sensitivity analysis is performed for TET removal predictions for the 180 L pilot HRAP. The set-points, with average, high, and low values are shown in Table 37, with the 'Normal' plot of fluctuating TET concentrations shown in Figure 45, and the sensitivity analysis shown in Figure 46, based on the response of TET removal predictions to the changes in the variables. The model predictions were most sensitive to changes in light intensity and the 'photodegradation yield' values (Figure 46).

Table 37: Average, high and low set-point values for sensitivity analysis.

Parameter	Units	'Average'	'High'	'Low'	Source of numbers
k_{1L}	10^{-5} L µmol^{-1}	1.2	4	0.3	Main text, Section 4.6
k_{1s}	d^{-1}	50	100	25	Main text, Section 4.3.2
K_d	L g^{-1}	2			Main text, Section 4.4.2
Intensity	µmol m^{-2} s^{-1}	Data from 13th Aug 2015	Data from 7th Aug 2015	Data from 14th Aug 2015	See Section S1.4.2 for graphed data
TSS	g L^{-1}	1.2	1	1.4	Main text, Section 4.1.1
evaporation rate	kg d^{-1}	10	12	8	Section S1.3.2
C_{in}	mg L^{-1}	0.1	0.11	0.09	10% error on the initial TET concentration
V	L	180	198	162	10% error on the volume of the 180 L HRAP
A	m^{-2}	1.2	1.32	1.08	10% error on the surface area of the 180 L HRAP

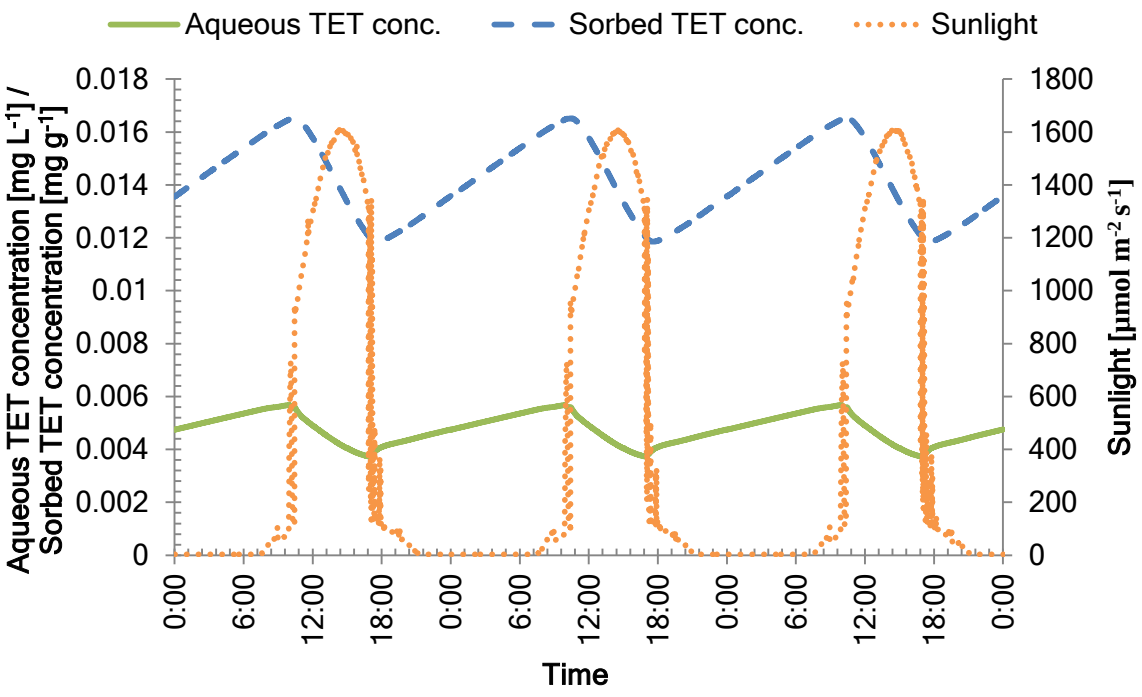

Figure 45: ‘Normal’ response for the continuous 180 L HRAP for the sensitivity test

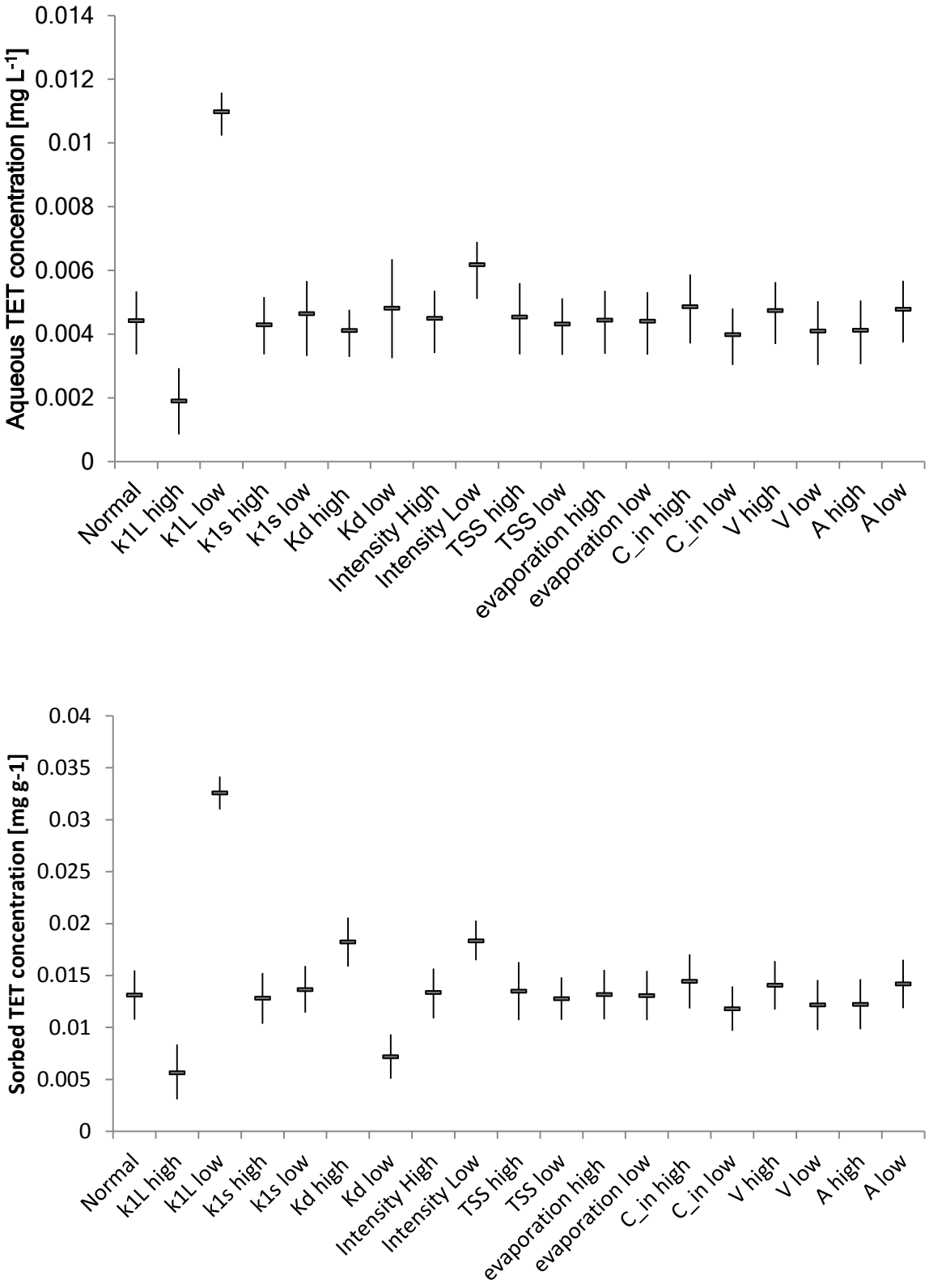

Figure 46: Sensitivity analysis for the continuous fate of TET in the 180 L HRAP, for both the aqueous and sorbed concentrations of TET, based on the parameters in Table 37.

S6. Appendix Six –Matlab code

In this section, the Matlab code is presented, as developed and used (Matlab version R2012b).

For all the model scripts, a script file is first used to set up the values for the different parameters, and set up the time period for modelling. Sudden changes (TET additions) split the time period into sub-sections, and each sub-section was then modelled by running the parameters set up with ode solvers (ode23s) and function files, then plotted. For sensitivity analysis, for-loops were used to cycle through different combinations of set-point parameter values, with statistics from the model simulation saved for comparison.

Data files (csv files) were used to load the recorded experimental concentrations of antibiotics already reported in this thesis, and the data-logged light intensity. The light-intensity data is not included here are the files are too large. Adaptations of the script file for different pond designs and to run the sensitivity analysis are not shown.

S6.1.1 Function files

This first function files includes the equations for the kinetic model used to predict TET removal under unsteady-state conditions, based on sorption and photodegradation

```
function Output=kineticsPhotodegradationSorption(t,D)
%Output=kineticsPilot(t,D) where D is a vector with [C q] and C is the
%aqueous antibiotic concentration and q is the sorbed concentration

global k1s k1L V Qin Qout C_in PAR Time k1ds TSS A SRT Kd

[~, idx] = min(abs(Time - t));
Light = PAR(idx)*3600*24;      %Convert umol/m2/s to umol/m2/d

one=-k1L*Light*A;      %first order constant L/d
%Units:-(L/d)-(L/umol)*(umol/m2/d)*(m2)

C=D(1);
q=D(2);
   dq=k1s*C/TSS-k1ds*q-q/SRT;
   %mg/g/d=1/d*(mg/L)/(g/L)-1/d*(mg/g)-(mg/g)*(1/d)
   dC=one*C/V+Qin*C_in/V-Qout*C/V-k1s*C+k1ds*q*TSS;
   %mg/L/d=L/d*mg/L/L+mg/d/L+L/d*mg/L/L-L/d*mg/L/L-mg/g/d*g/L
Output=[dC; dq];
```

A second function file was used to find matches the corresponding values from predicted TET concentrations and experimental sampling times for statistical analysis:

```
function YP=find_match(xE,xP,yP)
%Function YP=find_match(xE,xP,yP) xE and yE must be the same size, and xP
%and yP must be the same size.
%YP is the same length as xE, paired with the corresponding closest value of yP
%(it helps if there are many more data points for yP than yE)
idx=zeros(length(xE),1);
YP=zeros(length(xE),1);
for n=1:length(xE)
   [~,idx(n)]=min(abs(xP-xE(n)));
   YP(n)=yP(idx(n));
end
```

S6.1.2 Script file for running the model

```
%% Modelling HRAP
%Script file for plotting HRAP predictions of degradation with
%continuous flow with first order kinetic rates for sorption, desorption, and photodegradation
%loading datalogged light values from .csv files
%Applicable to both pulse tests and continuous flow
%Times (t variables) in DAYS by default
%Influent concentration C_in automatically stops after pulses start.
%Different Q in and Q out specified, to account for evaporation
clear all
close all
global k1s Kd k1L V Qin Qout C_in PAR Time k1ds TSS A SRT

%% Specify variables

%Kinetic Constants

k1L1=2.7e-5;    %Additional First Order Rate during light period    [L/umol]
k1L2=0e-5;%     %First order rate during light period on the second day  [L/umol]
k1s=15;       %Kinetic first order rate for sorption.            [d-1]
Kd=3;        %Sorption equilibrium constant                  [L/g]

V=900;       %Volume of HRAP [L]
Qin=100;  %L/d
Qout=90; %L/d
SRT=10;
A=3.5;       %Area of HRAP [m2]
%Influent Characteristics
C_in=2;     %mg/L

%% Choose dataset
Choose = 8; % Set which date range you want: 7th=1, 13th=2, 18th=3, 20th=4, 27th=5, 31st=6 (dates for
August, 180L HRAP in Spain);
          %    20th March=7, 25th March=8 for the 900L HRAP in NZ
clearpulses=0; %Set as 1 if you want to stop the Pulse additions


%% Data extraction

%Continuous conditions:

switch Choose
   case 1
PulseNum=2;        %Set number of pulses. If PulseNum is less than 2, variables will be ignored
accordingly
t_Pulse1 = datenum(2015,8,7,12,45,0);   %Time of first pulse (Y,MO,D,H,MI,S)
t_Pulse2 = datenum(2015,8,7,19,30,0);   %Time of second pulse (Y,MO,D,H,MI,S)
Load_p1=0.100*180;       %Load of tetracycline added in Pulse 1 in mg
Load_p2=0.100*180;       %Load of tetracycline added in Pulse 2 in mg
C_i=0.005;              %Initial concentration
TSS=1.1;      %Biomass concentration [g/L]
Data=xlsread('PARdata6thAugto8thAug.csv');
   case 2
PulseNum=2;        %Set number of pulses. If PulseNum is less than 2, variables will be ignored
accordingly
t_Pulse1 = datenum(2015,8,13,11,00,0);   %Time of first pulse (Y,MO,D,H,MI,S)
t_Pulse2 = datenum(2015,8,13,19,25,0);   %Time of second pulse (Y,MO,D,H,MI,S)
```

```
Load_p1=0.100*180;       %Load of tetracycline added in Pulse 1 in mg
Load_p2=0.100*180;       %Load of tetracycline added in Pulse 2 in mg
C_i=0.008;              %Initial concentration
TSS=1.36;     %Biomass concentration [g/L]
Data=xlsread('PARdata13thAugto14thAug.csv');
   case 3
PulseNum=1;       %Set number of pulses. If PulseNum is less than 2, variables will be ignored
accordingly
t_Pulse1 = datenum(2015,8,18,11,22,0);   %Time of first pulse (Y,MO,D,H,MI,S)
Load_p1=0.100*180;       %Load of tetracycline added in Pulse 1 in mg
C_i=0.003;              %Initial concentration
TSS=1.13;     %Biomass concentration [g/L]
Data=xlsread('PARdata17thAugto18thAug.csv');
   case 4
PulseNum=2;       %Set number of pulses. If PulseNum is less than 2, variables will be ignored
accordingly
t_Pulse1 = datenum(2015,8,20,10,25,0);   %Time of first pulse (Y,MO,D,H,MI,S)
t_Pulse2 = datenum(2015,8,20,19,30,0);   %Time of second pulse (Y,MO,D,H,MI,S)
Load_p1=0.100*180;       %Load of tetracycline added in Pulse 1 in mg
Load_p2=0.100*180;       %Load of tetracycline added in Pulse 2 in mg
C_i=0.01;              %Initial concentration
TSS=1.13;     %Biomass concentration [g/L]
Data=xlsread('PARdata20thAugto21stAug.csv');
   case 5
PulseNum=2;       %Set number of pulses. If PulseNum is less than 2, variables will be ignored
accordingly
t_Pulse1 = datenum(2015,8,27,10,55,0);   %Time of first pulse (Y,MO,D,H,MI,S)
t_Pulse2 = datenum(2015,8,27,19,05,0);   %Time of second pulse (Y,MO,D,H,MI,S)
Load_p1=0.100*180;       %Load of tetracycline added in Pulse 1 in mg
Load_p2=0.100*180;       %Load of tetracycline added in Pulse 2 in mg
C_i=0.018;              %Initial concentration
TSS=1.15;     %Biomass concentration [g/L]
Data=xlsread('PARdata27thAugto29thAug.csv');
   case 6
PulseNum=1;       %Set number of pulses. If PulseNum is less than 2, variables will be ignored
accordingly
t_Pulse1 = datenum(2015,8,31,19,30,0);   %Time of first pulse (Y,MO,D,H,MI,S)
Load_p1=0.100*180;       %Load of tetracycline added in Pulse 1 in mg
C_i=0.027;              %Initial concentration
TSS=1.05;     %Biomass concentration [g/L]
Data=xlsread('PARdata31stAugto1stSep.csv');
   case 7
PulseNum=1;       %Set number of pulses. If PulseNum is less than 2, variables will be ignored
accordingly
t_Pulse1 = datenum(2015,3,19,20,00,0);   %Time of first pulse (Y,MO,D,H,MI,S)
Load_p1=2*900;       %Load of tetracycline added in Pulse 1 in mg
C_i=0.1;              %Initial concentration
TSS=0.55;     %Biomass concentration [g/L]
Data=xlsread('PARdata19thMarto20thMarch.csv');
   case 8
PulseNum=1;       %Set number of pulses. If PulseNum is less than 2, variables will be ignored
accordingly
t_Pulse1 = datenum(2015,3,24,20,00,0);   %Time of first pulse (Y,MO,D,H,MI,S)
Load_p1=2*900;       %Load of tetracycline added in Pulse 1 in mg
C_i=0.05;              %Initial concentration
TSS=0.63;     %Biomass concentration [g/L]
Data=xlsread('PARdata24thMarto25thMarch.csv');
end
if clearpulses==1
   PulseNum=0;
```

```
end
%Desorption kinetic

k1ds=k1s/(Kd*TSS);        %Kinetic first order rate for desorption.          [d-1]

q_i=Kd*C_i*TSS;

%% Modify data extracted
time=Data(:,3);     %time expressed in days since 1900
kLux=Data(:,4);     %kLux values
PAR=Data(:,5);      %PAR, µmol/s.m2
t_exp=Data(:,6);
Tet_exp=Data(:,7);

%Remove NaN values from the end of the data
t_exp=t_exp(~isnan(t_exp));
Tet_exp=Tet_exp(~isnan(Tet_exp));

Time=time+693960;      %Time converted from Excel to Matlab datenumbers
t_exp=t_exp+693960;
Tet_exp=Tet_exp/1000;   %Convert from ug/L to mg/l

%% Set up the time sections
if PulseNum==0
   Time1=Time;
   timespan=length(Time1);
elseif PulseNum==1
   for set1=1:length(Time)
      if Time(set1)< t_Pulse1
         Time1(set1,1)= Time(set1);
         a=set1;
      else Time(set1)>= t_Pulse1;
         Time2(set1-a,1)=Time(set1);
      end
   end
   timespan(1)=length(Time1);
   timespan(2)=length(Time2);

elseif PulseNum==2
   for set1=1:length(Time)
      if Time(set1)<t_Pulse1
         Time1(set1,1)=Time(set1);
         a=set1;
      elseif Time(set1)>=t_Pulse1 && Time(set1) <t_Pulse2
         Time2(set1-a,1)=Time(set1);
         b=set1;
      else Time(set1)>= t_Pulse2;
         Time3(set1-b,1)=Time(set1);
      end
   end
   timespan(1)=length(Time1);
   timespan(2)=length(Time2);
   timespan(3)=length(Time3);
else
   error('PulseNum is not a recognised integer')
end

%% Numerical Solution
```

```
initial=[C_i, q_i];
options=odeset('MaxStep',0.01);
k1L=k1L1;
[t1, D1]=ode23s('kineticsPhotodegradationSorption',Time1,initial,options);
C1=D1(:,1);q1=D1(:,2);
if PulseNum>0
   C_in=0;
   initial=[C1(length(C1))+Load_p1/V, q1(length(q1))];
   [t2, D2]=ode23s('kineticsPhotodegradationSorption',Time2,initial,options);
   C2=D2(:,1);q2=D2(:,2);
end

if PulseNum==2
      k1L=k1L2;
   initial=[C2(length(C2))+Load_p2/V, q2(length(q2))];
   [t3, D3]=ode23s('kineticsPhotodegradationSorption',Time3,initial,options);
   C3=D3(:,1);q3=D3(:,2);
end


%% Plot
scsz=get(0,'ScreenSize');
figure('Position',[1 20 scsz(3) scsz(4)-110])
hold on
if PulseNum==0
plot(t1,C1,'b+');
plot(t1,q1*TSS,'b.')
end
if PulseNum==1
   plot(t1,C1,'b+',t2,C2,'g+');
   plot(t1,q1*TSS,'b.',t2,q2*TSS,'g.')
end

if PulseNum==2
   plot(t1,C1,'b+',t2,C2,'g+',t3,C3,'r+');
   plot(t1,q1*TSS,'b.',t2,q2*TSS,'g.', t3,q3*TSS,'r.')
end
NumXticks=8;
xticks=zeros(length(NumXticks));
for n=1:NumXticks
   xticks(n)=Time(round(1+(n-1)*(length(Time)/(NumXticks))));
end

xticks(NumXticks+1)=Time(length(Time));
Ylimits=get(gca,'Ylim');
set(gca,'XTick',xticks,'Ylim',[0, Ylimits(2)], 'FontSize', 22);
datetick('x', 'HH:MM','keepticks')
ylabel('Tetracycline Concentration [mg/L]')
% Plot experimental values

if PulseNum>0
   plot(t_exp,Tet_exp,'ko')
end
if PulseNum==0
   legend('Before Pulse eq.', 'Before Pulse Sorption')
elseif PulseNum==1
   legend('Before Pulse','Before Pulse Sorption eq.', 'Pulse 1', 'Pulse 1 Sorption eq.','Experimental Data')
elseif PulseNum==2
```

```
    legend('Before Pulse','Before Pulse Sorption eq.', 'Pulse 1', 'Pulse 1 Sorption eq.','Pulse 2','Pulse 2
Sorption eq.','Experimental Data')
end

hold off
%% create SSError
Date_plot=t1;
C_plot=C1;
q_plot=q1;
if PulseNum==1
Date_plot=[t1;t2];
C_plot=[C1;C2];
q_plot=[q1;q2];
end
if PulseNum==2
    Date_plot=[t1;t2;t3];
    C_plot=[C1;C2;C3];
    q_plot=[q1;q2;q3];
end


YPredict=find_match(t_exp,Date_plot,C_plot);
Residuals=YPredict-Tet_exp;
SSError=sum((Residuals).^2);
SS_tot=sum((Tet_exp-mean(Tet_exp)).^2);
R2=1-SSError/SS_tot;

sprintf('k1L1 = %0.2e; k1L2 = %0.2e; SSError = %0.4e; R^2 = %0.2f; Kd = %0.2e; k1s = %0.2e; k1ds =
%0.2e',k1L1,k1L2,SSError,R2,Kd,k1s, k1ds)

Plotting = [time, C_plot, q_plot];
t_exp=t_exp-693960;
PlotResiduals = [t_exp,Residuals,Tet_exp,YPredict];
```