Chemical Removal in Waste Stabilisation Pond Systems of Varying Configuration

Kathryn L. Linge[a,b], Deborah Liew[a], Yolanta Gruchlik[a], Francesco Busetti[a,c], Una Ryan[d], Cynthia A. Joll[a]

[a]*Curtin Water Quality Research Centre, Curtin University, GPO Box U1987, Perth, Australia*

[b]*ChemCentre, PO Box 1250, Perth, Australia*

[c]*School of Science, Edith Cowan University, Western Australia, 6027, Australia*

[d]*Vector- and Water-Borne Pathogen Research Group, School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch, Perth, Australia*

Corresponding author: K. Linge, Email: klinge@chemcentre.wa.gov.au, Tel: +61 (0)8 9422 9980, Fax: +61 (0)8 9422 9801

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Abstract

While nutrient removal has been well studied in waste stabilisation ponds (WSPs), studies of organic micropollutant removal in pond systems are limited. In this study, we investigated organic micropollutant, nitrogen and organic carbon removal in selected WSPs that differed in geographical location and pond configuration, and compared their performance to an oxidation ditch wastewater treatment plant (WWTP). Of the 232 chemicals measured, 36 were detected in wastewater influent and 33 were detected in treated wastewater effluent. New data for micropollutant removal in WSPs was generated for three pesticides or related chemicals, five pharmaceuticals, the plasticizer *N*-butylbenzenesulfonamide, the antioxidant 2,6-di-*t*-butyl-*p*-cresol, and two flame retardants tris(dichloropropyl)phosphate and tris(chloropropyl)phosphate isomers. Most of these micropollutants were relatively well removed in WSPs. The poorest treatment efficiency was observed in the single facultative pond system, with no maturation pond, suggesting that the presence of a maturation pond is important for chemical removal. The two WSPs in temperate climates were found to have higher concentrations of motile algae that can optimise their position with respect to light and temperature. However, to-date, the micropollutant removal by these algal species is not

known. The highest removals of micropollutants in a WSP were measured in a complex WSP system with two maturation ponds, and removals achieved were comparable to the oxidation ditch system. The key factors contributing to high micropollutant removal in this WSP were high solar irradiation and warm temperatures that promoted the growth of non-motile green algae previously found to degrade micropollutants, and photodegradation.

1. Introduction

Waste stabilisation pond (WSP) wastewater treatment systems are comprised of large shallow ponds that utilise natural physical and biological processes to remove organic matter, pollutants and pathogens present in raw wastewater.[1] The function of each pond is typically determined by its dimensions, in particular its depth. For example, facultative ponds, which range in depth from 1 m to 2.5 m are stratified systems that combine an aerobic surface layer and a bottom sludge-rich layer where anaerobic decomposition processes dominate. Facultative ponds are designed to remove biological and chemical oxygen demand and nutrients although pathogens can also be partially removed.[1] Maturation ponds, which follow the facultative ponds in conventional WSP systems, are shallow basins (1 – 2 m deep) in which aerobic conditions are maintained over the whole depth of the pond. While the main function of the maturation pond is the removal of pathogens, they can also contribute significantly to nutrient removal.[1]

Many researchers have investigated the mechanisms and pathways of nitrogen removal within WSPs and the principal mechanisms for nitrogen removal in WSPs include: ammonia volatilisation, nitrification and denitrification, and algal/microbial uptake and sedimentation.[2-4] The mechanisms that prevail in any given WSP depend strongly on environmental and operational conditions.

In contrast, studies of organic micropollutant removal in pond systems are less prevalent. For example, our recent review has shown that out of the many hundreds of organic micropollutants identified in wastewater and in wastewater impacted environments, only a limited number of

56 micropollutants (approximately 40) have been studied in WSPs,[5] representing a significant
57 knowledge gap in this area. Most of the compounds studied in WSPs to date have been
58 pharmaceuticals and personal care products (PPCPs) or endocrine disrupting compounds (EDCs).[5]
59 Like most wastewater treatment processes, WSPs are not designed for micropollutant removal.
60 However, reported removal mechanisms for organic micropollutants in WSPs include
61 photodegradation, biodegradation and sorption onto organic matter or sludge.[5, 6] Removal of organic
62 micropollutants is also influenced by many factors, such as the type and configuration of the ponds,
63 the operational parameters of the treatment plant, the wastewater quality, environmental factors (e.g.
64 sunlight, temperature, redox conditions and pH), as well as the chemical and physical properties of
65 the micropollutant.[5, 6]

66 The main removal mechanisms for micropollutants in WSPs are sorption, biodegradation and
67 photodegradation.[5] In WSPs, biodegradation is influenced by reciprocal biological interactions
68 between algae and microbial communities. For example, algal photosynthesis provides oxygen and
69 organic exudates which can then be used by pollutant-degrading bacteria.[7] Algae can also actively
70 participate in micropollutant biodegradation, as enzymes produced by algae can transform
71 micropollutants in a similar manner to detoxification reactions undertaken by mammalian livers.[8] The
72 degree of micropollutant removal depends on the algal species, with many studies undertaken with
73 green microalgae.[9] Because algae growth is typically controlled by sunlight, the effect of
74 photodegradation cannot necessarily be distinguished from algal degradation.[10]

75 While photodegradation upon exposure to sunlight is considered a key mechanism for micropollutant
76 removal in WSPs, only limited studies have been published.[5] Photodegradation can occur either by
77 direct absorption of light by the contaminant, or indirectly facilitated by reactive species (e.g.
78 hydroxyl radicals or singlet oxygen) produced by photosensitisers present in the water, such as nitrate
79 and nitrite or chromophoric organic matter.[11] Both direct and indirect photolysis have been identified
80 as micropollutant removal mechanisms in WSP.[12, 13] Micropollutants that are most likely to undergo

photodegradation will contain aromatic rings, heteroatoms and functional groups (e.g. phenol and nitro groups) that allow direct absorption of solar radiation, or react with reactive species.[14]

Sorption onto sludge or particulate matter is facilitated by hydrophobic interactions between hydrophobic pollutants and sludge solids, and electrostatic interactions between positively charged micropollutants and negatively charged microorganisms and effluent organic matter.[15] Sorption can be measured by the octanol-water partition coefficient (log K_{OW}) or the equilibrium partition coefficient for sorption onto activated sludge ($K_{d\text{-}AS}$, L kg^{-1}) which is typically defined for equilibrium conditions in a batch reactor.[16, 17] Only micropollutants with $K_{d\text{-}AS}$ > 500 L kg^{-1} have significant sorption onto activated sludge in municipal WWTPs.[17]

Reuse of treated wastewater for non-potable purposes is becoming an important water management strategy in many parts of regional Australia. However, there is still little information regarding the concentration of organic micropollutants in wastewater in regional areas, or their removal in WSP systems. The aim of this study was to assess organic micropollutant removal in selected WSPs and compare their performance to an oxidation ditch wastewater treatment plant (WWTP). The oxidation ditch is a modified activated sludge biological treatment process that uses long solids retention times to remove biodegradable organic compounds, typically configured in a concrete oval, or 'racetrack' configuration.[18] The selected WSP systems differed in geographical location, climate and design. The overall treatment performance was also assessed by considering nitrogen and dissolved organic carbon (DOC) removal in each system. The chemical removal results were also interpreted in combination with results from 16S and 18S next generation sequencing, which provided an overview of the bacterial and eukaryotic composition in each WWTP.

2. Materials and methods

2.1. Sampling and sampling sites

Samples were collected from three waste stabilisation ponds (WWTPs 1, 2 and 3) and an oxidation ditch (WWTP 4) in Western Australia (WA), all treating domestic wastewater. A description of the sampling sites and the sampling points is presented in Table 1, while general climatic data for each

location is provided in the Supporting Information (SI) Table S1. The WSP systems studied each consisted of a facultative pond, with varying numbers of maturation ponds (see SI Figure S1). Additionally, WWTP 1 utilised an advanced facultative pond, which consisted of a deep (~4 m) anaerobic digester pit overlaid with a facultative pond.[19] The advanced facultative pond is designed to optimise sedimentation and anaerobic degradation of wastewater organic solids.[1, 19]

Samples were collected from WWTP 1 during the wet and dry season, while samples from WWTPs 2, 3, and 4 were collected during summer and winter. Due to limitations of equipment, and lack of power at some sites, all samples were taken as grab samples. Field measurements were recorded for each sample using portable meters for pH, conductivity, temperature and dissolved oxygen (HQ40d, Hach Co., USA).

Table 1
Description of sampling sites and sampling points.

Sample site	Location and climate	Treatment process	Sample points	Sampling dates
WWTP 1	North West region of WA with a tropical climate. Seasons: wet and dry.	Advanced facultative pond, followed by two maturation ponds	Influent WW Post-Combined Pond Treated WW	19 Feb 2015 and 7 Sept 2015
WWTP 2	Wheat belt region with hot dry summers and mild winters. Seasons: four distinct seasons.	One facultative pond	Influent WW Treated WW	12 Feb 2015 and 13 Jul 2015
WWTP 3	South-Western Australia with a temperate climate. Seasons: four distinct seasons.	Two primary facultative ponds, and one maturation pond	Influent WW Post-Facultative Pond Treated WW	23 Feb 2015 and 14 Jul 2015
WWTP 4	South-Western Australia with a temperate climate. Seasons: four distinct seasons.	Oxidation ditches followed by sedimentation tanks	Influent WW Treated WW	23 Feb 2015 and 14 Jul 2015

Samples were collected using appropriate containers, as described in SI Table S2. Sample bottles were filled to zero headspace, kept cool (in an ice box) and transported back to the laboratory. Samples were filtered through 0.45 μm membrane filters, and refrigerated in the dark at 4°C until analysis. Trip and field blanks containing ultrapure water and preservation agent were also included during each sampling event to determine if there was any contamination through the sampling process, storage and transport. Trip blanks remained unopened until analysis, and field blanks were opened at each sampling location.

2.2. Analytical standards and chemicals

Ultrapure water from an ELGA purification system (resistivity of 18 mΩ, total organic carbon (TOC) 1 μg/L) was used for preparation of solutions. All solvents and reagents used in this study were of analytical grade purity (AR grade ≥99% pure or better). Details of reagents and analyte chemicals are provided in SI Table S3.

2.3. Chemical analyses

2.3.1 Nutrients and dissolved organic carbon

Water quality parameters measured by standard methods [20] included DOC (5310 B) using a Shimadzu high temperature combustion TOC-L analyser at Curtin University, and ammonia (4500-NH_3 G, LOR = 0.01 mg/L), nitrate and nitrite (4500-NO_3^- F, LOR = 0.01 mg/L), total nitrogen (4500-N C, LOR = 0.01 mg/L), total phosphorus (TP) (4500-P J, LOR = 0.01 mg/L) and sulphate (4500-SO_4^{2-} E, LOR = 1 mg/L) by a commercial laboratory. Due to the potential negative interferences of sulphides and thiols known to occur with the indophenol reaction for the ammonia measurement method (Phan et al., 1982), the sum of ammonia and organic nitrogen, also known as Kjeldahl nitrogen,[20] was considered as a combined parameter for data analysis, rather than the individual parameters of ammonia and dissolved organic nitrogen. Thus total Kjeldahl nitrogen (TKN) was determined as the difference between total nitrogen and the sum of nitrate and nitrite.

2.3.2 Organic micropollutants

A large suite of 232 organic micropollutants were analysed in the project. The analytical suite was derived from our previous studies of micropollutants in domestic wastewater and wastewater recycling.[21-27] In addition, due to the rural nature of some of the study sites, samples were analysed for a large suite of 159 pesticides and other chemicals by Queensland Health Forensic and Scientific Services (QHFSS, see SI Table S4).

Micropollutants analysed in-house at Curtin (SI Table S5) were measured using solid-phase extraction (SPE) followed by liquid chromatography-mass spectrometry (LC-MS) using validated analytical methods developed and published by our group and included pharmaceuticals,[26] antibiotics,[24] benzotriazole and benzothiazole corrosion inhibitors,[22] iodinated contrast media[25] and artificial sweeteners.[28] Chemicals were quantified using certified standards and deuterated internal standards. Where deuterated homologues were not available for a compound, suitable deuterated standards were chosen to represent groups of compounds.

3. Results and discussion

3.1. Assessment of WWTP treatment efficiency using nitrogen, DOC, and other bulk parameters

Wastewater (WW) was characterised by its bulk parameters, as described in SI Table S6 (field measurements) and Table S7 (nutrients and DOC), with a summary provided in Table 2. These bulk parameters were at concentrations typical for untreated municipal wastewater.[29]

Table 2
Concentration of nitrogen species and DOC for all sites, over two sampling events.

WWTP[a]	Sample point	Nitrite (mg N/L)	Nitrate (mg N/L)	TKN (mg N/L)	TN (mg N/L)	DOC (mg C/L)	%TN Removal	%DOC Removal
Sampling Event E1 (Summer or Wet Season)								
1	Influent WW	0.01	0.02	95.0	95	52		
	Post-combined Pond	0.2	0.03	60.8	61	9		
	Treated WW	0.07	0.08	20.9	21	11	78%	79%
2	Influent WW	0.09	0.02	42.9	43	24		
	Treated WW	0.08	0.02	64.9	65	22	-51%	7%
3	Influent WW	<0.1	0.07	99.9	100	41		
	Post-facultative Pond	<0.1	0.3	71.7	72	27		
	Treated WW	0.5	1	58.5	60	22	40%	46%
4	Influent WW	<0.1	0.08	99.9	100	25		
	Treated WW	0.2	3.1	0	3.2	6	97%	75%
Sampling Event E2 (Winter or Dry Season)								
1	Influent WW	0.01	<0.01	46.0	46.0	80		
	Post-combined Pond	<0.01	<0.01	49.0	49.0	13		
	Treated WW	0.01	<0.01	33.0	33.0	20	28%	75%
2	Influent WW	<0.01	<0.01	46.0	46.0	16		
	Treated WW	0.04	0.01	46.0	46.0	17	0%	-3%
3	Influent WW	0.02	<0.01	54.0	54.0	33		
	Post-facultative Pond	0.06	<0.01	46.9	47.0	20		
	Treated WW	<0.01	<0.01	40.0	40.0	21	26%	36%
4	Influent WW	<0.01	<0.01	79.0	79.0	33		
	Treated WW	0.11	0.93	1.5	2.5	7	97%	77%

[a] WWTP 1 is in a tropical region and was sampled in the wet and dry seasons, while WWTPs 2-4 are located in temperate regions and were sampled in summer and winter.

Based on removal of DOC and TN (Table 2), the efficiency of chemical removal followed in the order of: oxidation ditch system (WWTP 4) ⩾ advanced facultative pond system (WWTP 1) > simple pond systems (WWTP 2, WWTP 3). WWTP 4 had the best TN removal (97% in both events), followed by WWTP 1 (up to 78% removal), WWTP 3 (up to 40% removal), and finally WWTP 2 (0% removal or lower). The efficacy of N removal in WWTP 4 is attributed to optimised nitrification and denitrification in this oxidation ditch WWTP, with ammonia converted to nitrite and then to nitrate in the aerobic oxidation ditch, and nitrate is converted to nitrogen gas in an anoxic tank that receives recirculated mixed liquor from the oxidation ditch.[30]

WWTP 1 and WWTP 3 had greater TN removals during the summer/wet season (78% and 40%, respectively) than in the winter/dry season (28% and 26%, respectively), while WWTP 4, the oxidation ditch, exhibited similar TN removals over both summer and winter. WWTP 2 had no TN removal in winter (0%), and negative removal in summer (-51%). Nitrogen removal in WSPs could occur via nitrification and denitrification or through algal/microbial uptake and sedimentation, with the relative importance of each process changing seasonally, depending on the phytoplanktonic activity.[2, 3, 31]

The removal of DOC generally followed a similar trend to TN removal, but was not as affected by season as TN. Both WWTP 1 and WWTP 4 had similar DOC removals during both seasons (75-79%), while WWTP 3 and WWTP 2 had slightly higher DOC removals during summer (46% and 7%, respectively) than winter (36% and -3%, respectively). For WWTP 1 and WWTP3, most DOC removal occurred in the primary facultative pond, rather than the maturation ponds, and this agrees with typical primary facultative pond function, where organic matter may be oxidised by aerobic and facultative bacteria, as well as digested in anaerobic processes.[1] The higher DOC removal observed for WWTP 1 is likely due to its advanced facultative pond, which incorporates a deep anaerobic pit designed to optimise sedimentation and anaerobic degradation of wastewater organic solids.[32] However, temperature also influences anaerobic waste degradation in WSPs, with increased

degradation occurring at higher temperatures.[33] The DOC removals reflect the temperature patterns observed for each WSP (SI Table S1), where the monthly maximum temperature for WWTP 1 was similar for each sampling event (Event E1: 32.9 °C, E2: 31.9 °C), compared to the WSPs in temperate climates where summer temperatures (WWTP 2: 33.6 °C, WWTP 3: 29.6 °C) were much higher than winter (WWTP 2: 16.9 °C, WWTP 3: 17.5 °C).

For WWTP 1 and WWTP 3, greater TN removal occurred in the maturation ponds than in the primary facultative pond (Table 2). Maturation ponds contribute significantly to nutrient removal via algal biomass production.[31, 34] Algal productivity in WSPs can be indicated by changes in water pH, consumption of carbon dioxide via photosynthesis typically leading to increased pH.[35] Kayombo *et al*.[36] studied diurnal fluctuations of pH and DO in a pond system and concluded that pH > 8 indicated that photosynthesis was consuming more carbon dioxide (CO_2) than produced by bacterial respiration or organic matter decomposition, while pH < 8 indicated that photosynthesis did not completely utilize carbon dioxide. The rate of increase and decrease of DO in the WSPs also followed the same pattern as pH.[36]

The pH of influent entering all three WSPs was between 7.58 and 7.9 (SI Table S6), with DO concentrations typically below 2 mg/L. The pH remained similar after primary facultative pond treatment (pH 7.32-7.94), but increased after maturation pond treatment. Significant algal production (i.e. indicated by pH > 8) in maturation ponds was observed for WWTP 1 for both sampling events (E1: 9.2, E2: 8.47), and for Event 1 (summer, 8.66) for WWTP 3, which was also when the largest increases in DO concentrations were observed (SI Table 6). Sunlight provides the energy source for photosynthesis,[37] and WWTP 1 experienced consistent solar irradiation for each sampling event (E1: 21-24 MJ/m^2, E2: 24-27 MJ/m^2), compared to WWTP 3, where solar irradiation was significantly lower in Event 2 (E1: 24-27 MJ/m^2, E2: 9-12 MJ/m^2).

Both WWTP 1 and WWTP 3 had greater TN removals during Event 1 (78% and 40%, respectively) than in Event 2 (28% and 26%, respectively). For WWTP 3, pH and DO concentrations indicate that

algal production was higher during Event 1 than Event 2, linked to the significant difference in solar irradiation and temperature between summer and winter sampling events (SI Table S1). WWTP 1 also showed evidence that algal production was higher during Event 1 (wet season, pH = 9.2, DO = 16.2 mg/L) than during Event 2 (dry season, pH = 8.47, DO = 9.69 mg/L). However, solar irradiation and temperature were similar for both events (SI Table S1), with the main climatic difference being the average rainfall for each sampling month (E1: 181.0 mm, E2: 1.4 mm). Storm events can impact WSP function by changing organic or hydraulic loading, though this typically affects the anerobic treatment process.[38] It is not clear how rainfall may have impacted algal biomass production in this study and further seasonal data would be required to understand this effect.

WWTP 2 had considerably poorer treatment efficiency than WWTP 1 or WWTP 3, with no TN removal in winter (0%), and negative removal in summer (-51%). WWTP 2 consists of a single primary facultative pond, and the pH of treated wastewater (E1: 7.42, E2: 7.94) indicated that algal production was likely low. However, there was a marked difference between DO concentrations for the two sampling events (SI Table S6). In Event 1, the DO concentration was 0.62 mg/L in influent wastewater and 0.22 mg/L in treated wastewater. In Event 2, the DO concentration was 4.67 mg/L in influent wastewater and 5.63 mg/L in treated wastewater. The difference in the DO concentrations in wastewater influent cannot be explained, but is potentially related to increased water flow in winter compared to summer. The increased DO concentration in influent wastewater in Event 2 may have resulted in better nitrification, and this is supported by the slight increases in nitrite and nitrate. Increased DO would potentially reduce anaerobic degradation, which may explain why DOC removal was better in Event 1. However, overall DOC and TN removal was not well controlled in WWTP 2, and this WSP may benefit from additional treatment ponds to improve nutrient removal.

The pH of influent for WWTP 4 was significantly higher (E1: 8.3, E2: 8.43) than the pH values obtained for influents to the WSPs (SI Table S6), and no reason is known for this. However pH in treated wastewater from WWTP 4 was much lower (E1: 7.77 E2: 7.48) than the pH of treated wastewater from the WSPs (SI Table S6), suggesting that excess CO_2 produced by bacterial

244 respiration was not consumed by photosynthesis, as expected for an oxidation ditch WWTP.[30] While
245 the DO concentrations did increase during wastewater treatment (<0.9 mg/L for wastewater influent
246 and >6 mg/L in treated wastewater, SI Table S6), this was due to the effect of mixing paddles designed
247 to increase aeration in the oxidation ditch, rather than a by-product of photosynthesis.

248 *3.2. Micropollutant removal during wastewater treatment*

249 Of the 232 chemicals measured in the wastewater samples, 36 were detected in wastewater influent
250 and 33 were measured in treated wastewater effluent. Maximum and minimum concentrations
251 detected in wastewater influent and treated wastewater are presented in SI Tables S8 and S9,
252 respectively.

253 The removal of organic micropollutants, DOC and TN for each WWTP is presented in SI Table S10,
254 calculated as the difference in concentrations between the influent and treated wastewater, as a
255 percentage of the influent concentration. In some instances, micropollutants were reduced to below
256 detection after treatment. In these cases, a value equal to half the limit of reporting was used as a
257 conservative estimate of the final concentration. Removals for individual organic micropollutants
258 varied widely (-2188% to 100%), including a significant number of negative removals, which
259 typically resulted from the organic micropollutant not being detected in wastewater influent, but
260 present at low concentrations in wastewater effluent. This is likely due to limitations of grab sample
261 collection, and the potential for very highly variable micropollutant concentrations in wastewater
262 influent, due to discrete packets of water entering the sewerage from toilet flushes or other
263 appliances.[39] It is also possible that negative removals of compounds may be due to the presence of
264 unquantified metabolites and/or transformation products (e.g. glucuronide, methyl and glycine
265 conjugates) which were then hydrolysed back to the parent compound during the wastewater
266 treatment process.[40] Due to the presence of negative removals, median removal was calculated for
267 each WWTP, rather than average, because the median is less affected by outlier values. This study
268 has generated new occurrence and removal data for a number of organic micropollutants not
269 previously measured in WSPs (SI Table S10), including three pesticides or related chemicals (DEET,

piperonyl butoxide and 4-chloro-3,5-dimethylphenol), five pharmaceuticals (morphine, phenytoin, atorvastatin, cotinine, warfarin), the plasticizer *N*-butylbenzenesulfonamide, the antioxidant 2,6-di-*t*-butyl-*p*-cresol (BHT), and the flame retardants tris(dichloropropyl) phosphate and tris(chloropropyl) phosphate isomers.

Despite the large variation, the median percentage removal over all micropollutants was similar to the removal observed for DOC and TN (Table 2), with the order being WWTP 1 (E1: 94%, E2: 93%) > WWTP 4 (E1: 91%, E2: 75%) > WWTP 3 (E1: 82%, E2: 35%) > WWTP 2 (E1: 19%, E2: 0%). Median micropollutant removal was higher in summer than in winter for the WSPs (WWTP 2 and WWTP 3) and the oxidation ditch plant (WWTP 4). However, WWTP 1 had consistent micropollutant removal over both sampling events, in the wet season and dry season where temperature and solar irradiation were much more similar (SI Table S1). Higher micropollutant removal in WSPs has previously been observed in summer due to higher temperatures, greater biological activity and increased solar irradiation.[5, 7, 41] Temperature has also been identified as a key control parameter for treatment efficacy in oxidation ditch WWTPs.[42] Generally, enhanced micropollutant removal in WWTPs is achieved at warmer temperatures due to promotion of microbial activities.[43]

3.3. Comparison of micropollutant removal in WSPs and the oxidation ditch

Table 3 compares median removal of micropollutants in all WSPs (WWTPs 1-3), to the oxidation ditch (WWTP 4), and previously reported removals in WSPs. Only micropollutants detected in more than 50% of wastewater influent samples were included in the comparison, as otherwise removal values were affected by non-detects. Eleven of the 22 micropollutants in Table 3 were well removed (>75% removal) in both the WSPs and the oxidation ditch. Micropollutants from this group which have been previously studied in WSPs (ibuprofen, naproxen, triclosan, galaxolide, and sulfamethoxazole) showed similar high removals (Table 3).

Four chemicals (carbamazepine, benzotriazole, 4+5-methylbenzotriazole and sucralose) had less than 25% removal in both the WSPs and the oxidation ditch. These chemicals have been typically found

296 to be recalcitrant in wastewater treatment.[22, 44, 45] Removals previously reported for carbamazepine
297 and sucralose in WSPs are similar (Table 3). A single study[46] did find higher removals for
298 benzotriazole and 4+5-methyl benzotriazole, apparently related to the very good treatment efficiency
299 in this WSP, attributed to coexistence of different removal mechanisms, such as biodegradation,
300 sorption and photodegradation, and the presence of microalgae in both the cold and warm season.

301 DEET, gemfibrozil, *N*-butylbenzenesulfonamide, and trimethroprim had much better removal in the
302 oxidation ditch (72-97%) than in the WSPs (-214% to 56%). Neither DEET nor *N*-
303 butylbenzenesulfonamide have been previously measured in WSPs, but gemfibrozil has been found
304 to be poorly removed (15-20%) in other WSP studies.[47, 48] The removal of trimethoprim in WSPs has
305 also been found to be variable.[41, 49] Tonalide, warfarin, and tris(chloropropyl) phosphate isomers all
306 had slightly better removal in the WSPs than the oxidation ditch, with similar removal of tonalide
307 compared to other WSP studies,[46, 49] and better removal of tris(chloropropyl) phosphate isomers.[46]

308 **Table 3**
309 Comparison of median removal of micropollutants in all WSPs (WWTP 1-3), with median removals
310 in the oxidation ditch (WWTP 4), and previously reported WSP studies. Only micropollutants
311 detected in more than 50% of wastewater influent samples were included in the comparison.

	This study		Previously Published Values	
	WSPs	Oxidation Ditch		
>75% Removal in WSPs and Oxidation Ditch				
Ibuprofen	79%	100%	75-100%	41, 46-50
Naproxen	83%	99%	75-100%	41, 46-48, 50
Cotinine	97%	99%		
Triclosan	89%	99%	77-100%	41, 46, 48, 49
Morphine	82%	99%		
4-Chloro-3,5-dimethylphenol	82%	99%		
Atorvastatin	87%	95%		
2,6-Di-*t*-butyl-*p*-cresol	93%	94%		
Piperonyl butoxide	91%	89%		
Galaxolide	84%	80%	75-96%	46, 49

Sulfamethoxazole	94%	75%	82-100%	41, 47, 49
Variable Removal				
DEET	56%	97%		
Gemfibrozil	-214%	95%	15-20%	47, 48
N-Butylbenzenesulfonamide	-50%	86%		
Trimethoprim	36%	72%	44-100%	41, 49
Tonalide	87%	70%	76-96%	46, 49
Warfarin	65%	61%		
Tris(chloropropyl) phosphate isomers	64%	40%	21%	46
< 25% removal in WSPs and Oxidation Ditch				
Carbamazepine	20%	-41%	6-29%	46-48
Benzotriazole	10%	1%	50%	46
4+5-Methyl Benzotriazole	2%	25%	76%	46
Sucralose	1%	4%	Not removed	49

3.4. Mechanisms of micropollutant removal in WSPs and the oxidation ditch

Sorption, biodegradation and photodegradation were assessed for their likely role in micropollutant removal observed in the WWTPs in this study. The contribution of sorption to micropollutant removal was assessed by considering the octanol-water partion coefficient, log K_{OW},[51] and the equilibrium partition coefficient for sorption onto activated sludge, $K_{d\text{-}AS}$ (L kg^{-1}), summarised in SI Table S11. Biodegradation was assessed by considering experimentally determined pseudo first-order rate constants for biodegradation in activated sludge ($k_{biol\text{-}AS}$) (SI Table S11). While photodegradation upon exposure to sunlight is considered a key mechanism for micropollutant removal in WSPs, only limited studies have been published.[5, 52] The rate of photodegradation will be impacted by the mode of degradation (direct or indirect), the presence of photosensitisers in the water, and whether the micropollutant contains aromatic rings, heteroatoms and functional groups (e.g. phenol and nitro groups) that allow direct absorption of solar radiation. Of the micropollutants listed in Table 3, all except sucralose and the tris(chloropropyl) phosphate isomers contain an aromatic ring, while all micropollutants contain a heteroatom other than C or H, suggesting most of these micropollutants may be amenable to photodegradation.

When considering the contribution of sorption, triclosan, galaxolide, and tonalide were the only chemicals with $K_{d\text{-}AS}$ > 500 L kg^{-1}. All of these micropollutants also have log K_{OW} > 4, which has also been proposed as an indicator for strong sorption onto sewage sludge.[53] Galaxolide and tonalide are both known to be predominantly removed by sorption in activated sludge WWTPs[15] and have also been reported to be sorbed to the biomass (i.e. algae) in high rate algal ponds.[7] Triclosan has been found to be strongly sorbed to sludge,[54] although biodegradation will also contribute to its removal.[15, 55]

Six compounds in this study have not had $K_{d\text{-}AS}$ determined previously (SI Table S11). Tris(1-chloro-2-propyl) phosphate, 4-chloro-3,5-dimethylphenol and *N*-butylbenzenesulfonamide all have log K_{OW} < 4, suggesting that minimal sorption onto wastewater sludge is expected, and this is agreement with the limited studies of these compounds. Less than 5% of dissolved tris(1-chloro-2-propyl) phosphate was found to sorb on suspended particulate matter in wastewater,[56] while no sorption of 4-chloro-3,5-dimethylphenol onto sludge was identified in abiotic bioreactors.[57] Piperonyl butoxide, 2,6-di-*t*-butyl-*p*-cresol (BHT) and atorvastatin all have log K_{OW} > 4. Sorption of atorvastatin (log K_{OW} = 6.36) is limited because it is negatively charged in wastewater, which reduces sorption onto activated sludge. Studies of piperonyl butoxide and 2,6-di-*t*-butyl-*p*-cresol (BHT) have found greater removal via biodegradation than sorption.[8, 58]

Microbial biodegradation may occur via mineralization as a carbon and energy source for bacteria, as part of a mixed substrate, or via co-metabolism, in which degradation is facilitated by enzymes generated for other primary substrate degradation.[43] Previous modelling[59] has suggested that micropollutants with k_{biol} < 0.1 L g^{-1} d^{-1} are removed less than 20% by biodegradation in conventional wastewater treatment plants, while micropollutants with $k_{biol\text{-}AS}$ = 1 L g^{-1} d^{-1} would be biodegraded by 70-80%, and micropollutants with $k_{biol\text{-}AS}$ > 10 L g^{-1} d^{-1} will have more than 90% removal by biodegradation.

Of the eleven compounds with >75% removal in both the WSPs and the oxidation ditch, five (ibuprofen, naproxen, cotinine, morphine and galaxolide) have $k_{biol\text{-}AS} > 10$ (SI Table S11). Removal of ibuprofen, naproxen, cotinine, and morphine were all greater than 99% in the oxidation ditch, with relatively lower removal (79%-97%) observed in the WSPs. This suggests that the microbial community developed in the oxidation ditch is optimised for removal of these compounds. Removal of ibuprofen and naproxen in conventional wastewater treatment has been found to be predominantly through biodegradation.[15] Removal of galaxolide in the oxidation ditch was lower (80%), however the reported values of $k_{biol\text{-}AS}$ for galaxolide vary widely (0.03-170 L g^{-1} day^{-1}, SI Table S11), suggesting that biodegradation of galaxolide can be variable.

Of the remaining compounds with >75% removal, triclosan, atorvastatin and 4-chloro-3,5-dimethylphenol have reported $k_{biol\text{-}AS}$ values between 1.2 and 3.6, which suggest removal of >70-80% by biodegradation.[59] All of these micropollutants had >95% removal in the oxidation ditch, which may suggest additional removal mechanisms existed. As discussed earlier, triclosan removal occurs by both sorption and biodegradation.[15, 55] Biodegradation of atorvastatin has been found to be promoted in the presence of additional substrates to increase biological activity.[60] Neither 2,6-di-*t*-butyl-*p*-cresol nor piperonyl butoxide have $k_{biol\text{-}AS}$ values reported (SI Table S11), but studies support greater removal via biodegradation than sorption for both chemicals.[8, 58]

Despite >75% removal in both WSPs and the oxidation ditch and biodegradation being previosuly considered as the major removal mechanism in activated sludge wastewater treatment,[15] sulfamethoxazole has low (<1) $k_{biol\text{-}AS}$ values (SI Table 11). However, Ianaiev[61] suggested that conversion of metabolites (e.g. *N*-4-acetylsulfamethoxazole and sulfamethoxazole-glucuronide) back to sulfamethoxazole in wastewater through deconjugation reactions could artificially reduce measured $k_{biol\text{-}AS}$ values.

Of the seven compounds with removals between 25% and 75%, only tonalide had values of $k_{biol\text{-}AS} > 10$ (SI Table S11), although much lower values of $k_{biol\text{-}AS}$ for tonalide have also been reported.

Coupled with $K_{d\text{-}AS}$ > 500, tonalide removal should be more comparable to galaxolide removal, with both chemicals reported to have similar removal (85%) in activated sludge treatment.[15] The only other chemicals with reported values of $k_{biol\text{-}AS}$ are gemfibrozil and trimethroprim (SI Table S11). Gemfibrozil $k_{biol\text{-}AS}$ has been reported between 0 and 10, and biodegradation is the predominant removal mechanism in conventional WWTP[15] which is in agreement with the 95% removal found in the oxidation ditch in this study. However, removal in the WSPs was negative (-214%). Gemfibrozil has been found to be predominantly excreted in urine as an acyl glucuronide conjugate[62] and this metabolite can be hydrolysed back to the parent compound during wastewater treatment, resulting in apparent formation.[63, 64] Values of $k_{biol\text{-}AS}$ for trimethoprim are < 0.24, and the relatively low rates of removal measured in both the WSPs and the oxidation ditch (Table 3) agree with previous studies indicating that trimethoprim is more resistant to biodegradation than sulfamethoxazole.[65] Biodegradation was still likely to be the major degradation mechanism for trimethoprim, particularly in the oxidation ditch.[15]

Values of $k_{biol\text{-}AS}$ have not been reported for DEET, *N*-butylbenzenesulfonamide, warfarin or tris(chloropropyl) phosphate isomers. DEET, *N*-butylbenzenesulfonamide, and trimethoprim were much better removed in the oxidation ditch (72-97%) than in the WSPs (-214% to 56%), suggesting that biodegradation in the activated sludge was important for their removal. DEET has previously been found to be solely removed by biodegradation in activated sludge WWTPs.[15] In contrast, the removal of tris(chloropropyl) phosphate isomers was reported to be principally by sorption.[15]

All chemicals (carbamazepine, benzotriazole, 4+5-methylbenzotriazoles, sucralose) with low (<25%) removal in both WSPs and the oxidation ditch have $k_{biol\text{-}AS}$ < 0.5, with many reported values much lower than 0.1 (SI Table S11), indicating that biodegradation will not significantly remove these compounds. All of these chemicals also have $K_{d\text{-}AS}$ < 500, and thus sorption to solids is also expected to be very low, in agreement with previous assessments of removal of these chemicals in conventional wastewater treatment.[15]

Carbamazepine has been found to undergo photodegradation in wastewater, with a half-life of 31.5 hours.[13] This may explain the higher removal of carbamazepine in the WSPs (20%) compared to the oxidation ditch (-41%). The negative removal in the oxidation ditch is likely due to transformations of carbamazepine metabolites back to the parent pharmaceutical during wastewater treatment. In addition to carbamazepine, seven other micropollutants (piperonyl butoxide, galaxolide, sulfamethoxazole, tonalide, warfarin, tris(chloropropyl) phosphate isomers, and benzotriazole) had higher median removals in the WSPs than in the oxidation ditch. The contribution of photodegradation to removal in wastewater and aquatic systems has been confirmed for a number of micropollutants in Table 3, including sulfamethoxazole,[8, 12] atorvastatin,[52] carbamazepine,[52] naproxen[52], benzotriazole,[66] and tris(2-chloroisopropyl) phosphate.[67] Additionally piperonyl butoxide has been found to be degraded by sunlight, although rates of degradation were low.[68] The other chemicals with higher removal in WSPs have not yet been studied for photodegradation in wastewater systems. However, both galaxolide and tonalide can be removed by UV irradiation.[69], while warfarin has been shown to be degraded in TiO_2-catalysed UV and solar radiation experiments to a similar extent as carbamazepine, gemfibrozil and naproxen.[70]

3.5. Effect of biological community on chemical removal

In addition to pond configuration and climate, treatment efficiency is also influenced by the biological communities present in each WWTP. As well as the chemical analysis reported in this study, the bacterial and eukaryotic composition of each sample using next generation sequencing (NGS) has been previously reported, targeting the hypervariable region 4 (V4) of the bacterial 16S rRNA (16S) gene,[71] and the hypervariable 9 (V9) region of the eukaryotic 18S rRNA (18S) gene.[72] While genetic markers in water samples may be derived from both live and dead bacteria, NGS data indicated the presence of many organisms known to have specific functions in wastewater treatment.

Nitrification is a fundamental process for the biological removal of nitrogen in WWTPs, with ammonia converted to nitrite by ammonia-oxidising bacteria (AOB), and nitrite oxidised to nitrate by nitrite-oxidising bacteria.[33] Ammonia-oxidising bacteria were not detected in any WWTPs in this

428 study, while nitrite-oxidising bacteria (*Nitrospira* spp.) were only detected in the oxidation ditch and
429 effluent samples of WWTP 4. There are many reasons why nitrifying bacteria were not detected, even
430 in WWTP 4, where nitrification and denitrification are likely to be the major N removal mechanism.
431 AOB species may not have been efficiently amplified using the 16S V4 primers employed[71] and/or
432 the short amplicon size used for NGS may have produced unreliable taxonomic assignments for AOB
433 bacteria. Previous studies on microbial diversity of AOBs in wastewater using NGS have also
434 reported a low abundance of AOBs using the 16S V3-V4 region,[73] and suggest that species-specific
435 qPCR analyses may be required to better determine the AOB prevalence and diversity in these
436 WWTPs. While amplicon NGS is a useful tool for broad taxonomic surveys of bacteria, tools such
437 as quantitative PCR of functional genes that encode key enzymes may be useful to identify bacteria
438 that could not be differentiated at the species or strain level.

439 Nitrification is typically coupled with denitrification, where nitrate is reduced to nitrogen gas (N_2)
440 under anoxic conditions, enabling complete removal of nitrogen from the system. Denitrifying
441 bacteria were detected in all WWTPs, particularly the families Comamonadaceae and
442 Rhodocyclaceae. However, DNA from Comamonadaceae was mostly found in influent wastewater,
443 with between 14-21% of the total 16S DNA sequences (or sequence composition) measured in these
444 samples. This suggested these bacteria were already present in wastewater influent and were not part
445 of treatment processes in the WWTPs. The most common Comamonadaceae in the influents were
446 from the genus *Acidovorax* sp., an acid degrading proteobacteria known to exist in the human gut
447 microbiome.[74] The sequence composition of *Acidovorax* sp. decreased in all treated wastewater
448 samples, indicating that it did not grow in the WSPs or the oxidation ditch. Rhodocyclaceae sequence
449 compositions did increase with treatment for WWTP 3 (4% sequence composition in the influent and
450 29% in the treated wastewater) and WWTP 4 (4% in the influent and 18% in the treated wastewater).
451 Rhodocyclaceae are also linked to flocculation in activated sludge plants,[75] which is important for the
452 cultivation of floc-forming and fast settling microorganisms.[76] Floc-forming bacteria secrete
453 extracellular polymeric substances, which promote adhesion and interactions between

454 microorganisms and the various floc components. Thus the increasing sequence composition of
455 Rhodocyclaceae in WWTP 4 is consistent with sludge formation in an oxidation ditch WWTP. The
456 increasing sequence composition of Rhodocyclaceae observed in WWTP 3 suggested that floc-
457 forming bacteria also promoted sludge formation in this WSP, compared to WWTP 1 and WWTP 2.

458 DNA from the phylum Cyanobacteria comprised >11% sequence composition in treated wastewater
459 samples for all WSPs (WWTP 1: 19.2%, WWTP 2: 47.2%, WWTP 3: 11.7%), but only 0.7% in
460 WWTP 4 (the oxidation ditch). The main Cyanobacterial class in WWTP 4 (the oxidation ditch) was
461 4C0d-2, a non-photosynthetic organism which may predate on other bacteria.[77] The main
462 Cyanobacteria in the WSPs were filamentous species from the order Oscillatoriales. *Arthrospira*
463 *platensis* comprised 46.6% total bacterial sequence composition in WWTP 2, and was predominantly
464 detected in Event 1 (summer). DNA from the genus Planktothrix was found in treated wastewater
465 from WWTP 1 (14.2%) and WWTP 3 (2.6%). Neither Planktothrix or *A. platensis* fix nitrogen from
466 the atmosphere,[78, 79] suggesting they utilise the nitrogen in the wastewater and facilitate TN removal.
467 Both can use either ammonia or nitrate as a N source for algal biomass production.[80, 81] While the pH
468 and DO in WWTP 2 (SI Table S6) did not appear to indicate high algal productivity, a higher
469 sequence composition for *A. platensis* was observed in summer in WWTP2.

470 In addition to photosynthetic cyanobacteria, WWTP 1 had high sequence compositions in treated
471 wastewater of the eukaryotic phylum Chlorophyta (53.2% measured by 18S NGS), much higher than
472 all other WWTPs (<4% sequence composition). Chlorophyta is a phylum of typically unicellular
473 photosynthetic green algae that uptake nitrogen during production of algal biomass. The Chlorophyta
474 found in highest sequence abundance in WWTP 1 was *Chlorella sorokiniana,* which can use either
475 ammonia or nitrate as a N source for algal biomass production.[82] This and other *Chlorella* species
476 have also been found to remove a range of pharmaceuticals,[83] including carbamazepine, triclosan and
477 trimethoprim, which were all measured in this current study, with similar removals observed to those
478 reported in Table 3. The presence of *Chlorella sorokiniana* in WWTP1 may be linked to the better
479 micropollutant removal observed in this WSP, compared to the other temperate WSPs.

In contrast to WWTP 1, the dominant groups of eukaryotic phyla in the other WSPs (WWTP 2 and WWTP 3) and the oxidation ditch (WWTP 4) were Euglenozoa (20-38% sequence composition) and Ciliophora (23-51% sequence composition). The predominant genus for Euglenozoa present in the WWTP 2 and WWTP 3 was *Euglena*, a motile algae previously found to predominate in facultative ponds.[1] The top BLAST hit Euglenozoa gene in WWTP 4 (AB902317) could not be identified to species level but was previously reported in an oxidation ditch system in Japan.[84] *Euglena* can optimise their vertical position within the water column in relation to the incident light intensity and temperature,[1] which will ensure optimal growth in WWTP 2 and WTTP 3, where solar irradiation and temperature varied seasonally (SI Table S1). In contrast, WWTP 1 has relatively consistent temperature and solar irradiation throughout the year and non-motile algae like *Chlorella* are able to dominate. While *Chlorella* sp. are known to cause micropollutant degradation, to date, the micropollutant removal by *Euglena* sp has not been studied.

The phylum Ciliophora includes various classes of bacterivorous ciliates that are known to play a central role in wastewater clarification in activated sludge plants, by enhancing carbon mineralization by bacteria and grazing on suspended bacteria and particles.[85] Based on sequence composition, Ciliophora were more important for clarification in WWTP 2 (39.4% sequence composition) and WWTP 4 (51.1%), than WWTP1 (8.8%) and WWTP 3 (22.9%). Rotifers, a phylum of microscopic and near-microscopic pseudocoelomate animals, have also been shown to improve clarification in activated sludge WWTPs through consumption of biomass and enhanced aggregation.[86] However, they also graze on green microalgae,[87] which likely explains their higher presence in treated wastewater in the two WSPs with maturation ponds (WWTP 1, 9.5% and WWTP 3, 27.9%), compared to less than 2% in WWTP 2 and WWTP 4.

4. Conclusions

In this study, chemical removal in selected WSPs was compared to chemical removal in an oxidation ditch WWTP, with a particular emphasis on the removal of organic micropollutants. Similar treatment efficiencies were measured in a complex tropical WSP, with an advanced facultative pond followed

by multiple maturation ponds (WWTP1), and the oxidation ditch system. Poorest treatment efficiency was observed in the single facultative pond system with no maturation pond (WWTP 2), suggesting that the presence of a maturation pond was important for chemical removal.

Analysis of the bacterial and eukaryotic communities in each WWTP suggested that TN removal in WWTP 4 (the oxidation ditch) was *via* nitrification and denitrification, while TN removal in the WSPs was linked to algal productivity and the presence of photosynthetic cyanobacteria and eukaryotes. This interpretation was supported by changes in pH and DO that indicated increased algal productivity in the maturation ponds in WWTP 1 and WWTP 3. WSPs in temperate climates (WWTP 2 and 3) had higher concentrations of motile algae (*Euglena* sp.) that optimise their position with respect to light and temperature, compared to the tropical WSP with multiple maturation ponds (WWTP 1), where non-motile green algae (*Chlorella* sp.) predominated. *Chlorella* sp. are also known to cause micropollutant degradation, likely contributing to high micropollutant removal rates in WWTP 1.

Removal of micropollutants in WSPs in this study was comparable to previous studies, with new data for micropollutant removal in WSPs generated for three pesticides or related chemicals, five pharmaceuticals, the plasticizer N-butylbenzenesulfonamide, the antioxidant 2,6-di-t-butyl-p-cresol (BHT), and two flame retardants tris(dichloropropyl) phosphate and tris(chloropropyl) phosphate isomers. The similarity of micropollutant removal to other studies also suggested that considering median removal was an appropriate method of mitigating variability in grab samples. The main mechanism for micropollutant removal was proposed to be biodegradation, particularly for micropollutants with > 75% removal in both WSPs and the oxidation ditch. For chemicals that had better removals in the WSPs than the oxidation ditch (e.g. sulfamethoxazole, carbamazepine, piperonyl butoxide, warfarin, tris(chloropropyl) phosphate isomers, benzotriazole, galaxalide and tonalide), photodegradation in the WSPs is likely to have resulted in the additional removal.

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