Removal mechanisms of pharmaceuticals and personal care products during soil aquifer treatment

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ABSTRACT

Wastewater effluent is an alternative water resource to solve the water shortage in the world. Most pollutants can be removed effectively in wastewater treatment plants (WWTPs) by activated sludge (AS) treatment, but micropollutants are still detected in the effluents of WWTPs because of the limitation of wastewater treatments. The existence of micropollutants poses potential risks to human health and ecosystem. Thus, In order to improve the water quality of wastewater effluent, advanced treatments are necessary. Among advanced treatments, soil aquifer treatment (SAT) is widely used because it is simple, cost-effective, and environmental friendly. In this study, the removal mechanisms (i.e., sorption and biodegradation) of micropollutants in SAT and the cooperation of SAT and AS treatment were evaluated.

Among micropollutants, pharmaceuticals and personal care products (PPCPs) have a variety of chemical structures and physical chemical properties, so PPCPs can be indicators of micropollutants to evaluate their removal mechanisms in SAT. However, to my best knowledge, the removal mechanisms of PPCPs in SAT were not well understood in the previous studies. In this study, removal mechanisms of PPCPs in SAT and AS treatment were evaluated as well as the effects of operating conditions in SAT on the removals of PPCPs. Furthermore, transformations of caffeine and 2-arylpropionic acids (profens) were evaluated to understand the demethylation of micropollutants and the biodegradation pathways of carboxylic compounds in SAT and AS treatment, because of methyl functional groups in caffeine and carboxyl groups in profens. This thesis would be introduced as follows.

Chapter 3 presented the removal mechanisms of PPCPs in SAT and the comparison of biodegradation between SAT and AS treatment. The total concentration of 29 PPCPs in the effluents from the target WWTP (wastewater effluent) was up to approximately 5000 ng/L. Antibiotics, analgesics, and antilipidemics were dominant in the wastewater effluents. Firstly, sorption and biodegradation of PPCPs were investigated based on the difference of removals of PPCPs between the sterilized and non-sterilized columns. Levofloxacin, one of fluoroquinolone was removed by sorption because of its high affinity to organic compounds. Macrolides (lincomycin, azithromycin, and clarithromycin), triclosan, sulpiride, diltiazem, atenolol, and disopyramide were removed effectively by ionic interaction in SAT. Diclofenac, ketoprofen, naproxen, mefenamic acid, bezafibrate, clofibric acid, gemfibrozil, and furosemide, which possess carboxyl groups, were removed effectively in the non-sterilized SAT columns but not in sterilized SAT columns indicating their biodegradability in SAT. However, the removals of amide PPCPs (i.e., antipyrine, isopropylantipyrine, crotamiton, primidone, and carbamazepine) were poor in SAT. Secondly, soil batch-treatment experiments using autoclaved weathered granite soil (WGS) and WGS in SAT were operated to evaluate the influence of biomass on the sorption in SAT: the values of K_d of

fluoroquinolones in WGS in SAT were higher than those in autoclaved WGS because the growth of biomass increased the soil organic matter (SOM) in SAT. Thirdly, in lab-scale sequencing batch reactor (SBR) with the addition of biodegradable PPCPs, the half-lives of gemfibrozil, furosemide, clofibric acid and naproxen were longer than 6 hrs, and those of fenoprofen, ketoprofen, ibuprofen and bezafibrate were shorter than 6 hrs. From the perspective of chemical structure, furosemide, and clofibric acid possess halogens, and naproxen has a polycyclic aromatic structure, indicating the structures of halogen and the polycyclic aromatic suppressed the biodegradation rate of compounds in AS treatment. In soil batch-treatment experiment with the addition of biodegradable PPCPs, the biodegradation rate ranked as follows: fenoprofen, ibuprofen, clofibric acid, furosemide, naproxen, bezafibrate, gemfibrozil, and ketoprofen, which was different from the order in SBR. For evaluating biodegradation rate with respect to the amount of biomass, the $K_{A,X}$ values (the rate constants (K_A) with respect to biomass(X)) of naproxen, clofibric acid, and gemfibrozil were similar between these two treatments, and the $K_{A,X}$ values of other selected PPCPs in SBR were higher than those in SAT. These indicated that adaptable biodegradations for halogenated acid and polycyclic aromatic hydrocarbon (PAH) were formed in SAT. Fourthly, in the comparative study between SAT columns and SBR, clarithromycin and azithromycin were removed effectively in SBR, but the removal of lincomycin in SBR was moderate. Levofloxacin and sulpiride were removed poorly in SBR, indicating strong sorption is an advantage of SAT on the removals of micropollutants. Ketoprofen, naproxen, mefenamic acid, bezafibrate, clofibric acid, gemfibrozil, and furosemide were biodegradable in AS treatment as well as in SAT. However, diclofenac was effectively removed in SAT, but its removal was poor in SBR. The removal efficiency of caffeine in SBR was lower than that in SAT. For amide PPCPs, SBR showed poor removals on them similarly as SAT. For the practical purpose, SAT possesses a wider range of biodegradation than AS treatment, but the biodegradation was faster in AS treatment than in SAT. Thus, cooperation of AS treatment and SAT was suitable for the removals of micropollutant in wastewater reclamation.

Chapter 4 presented the effects of operating conditions in SAT on PPCP removals. Firstly, WGS obtaining high values of CEC, TOC and specific area was available for the sorption of antibiotics in SAT. The removals of biodegradable PPCPs were effective in the columns of WGS, SA and TS. For poorly biodegradable PPCPs, their removals were similarly poor in these columns. These indicated that packing materials have little influence on the characteristics of biodegradation in SAT. In WGS columns, the removal efficiencies of antibiotics were effective regardless HRT. Secondly, for biodegradable PPCPs (diclofenac, ketoprofen, naproxen, mefenamic acid, bezafibrate, clofibric acid, gemfibrozil, and furosemide), HRT of 7 days was sufficient for their removal efficiencies, and extending HRT from 7 days did not show clear improvement. The removals of sulfamethoxazole, theophylline and benzophenone were improved by extending HRT from 7 days. In this study, HRT of 7 days was efficient for the removals of most target PPCPs. Thirdly, the removals of antibiotics (except sulfamethoxazole), profens and antilipidemics were effective in the WGS column under the vadose condition as well

as under saturated condition. For poorly biodegradable PPCPs, such as primidone and carbamazepine, their removals were poor regardless vadose or saturated conditions. Sulfamethoxazole and crotamiton were removed more effectively under the vadose condition than under the saturated condition in SAT. This indicated that aerobic condition enhanced for their removals. Though aerobic condition improved the removal of sulfamethoxazole and crotamiton in SAT, saturated condition was sufficient on the removals of most target PPCPs. From the perspective of practical use, short HRT and saturated condition can increase the capacity of SAT. Overall, SAT using WGS with an HRT of 7 days under saturated condition could be a practical choice for controlling micropollutants in SAT.

In Chapter 5, transformations of caffeine and profens in SAT and AS treatment were evaluated. Among detectable caffeine transformation products (TPs), theophylline (TEP), 1-methylxanthine (1-MX), and xanthine (XAN) were detected at a higher concentration than caffeine in the wastewater effluent. AS treatment and SAT were similar on the caffeine demethylation products: TEP and theobromine (THB) were primary TPs; 1-MX and 7-methylxanthine (7-MX) were secondary TPs; Xanthine was tertiary TP. SAT has the advantage on the degradation of multi-methyl xanthines in comparison with AS treatment. Furthermore, SAT with a long HRT degraded CAF and its TPs more effectively than AS treatment, but SAT with a short HRT had the risk on increasing the concentration of TPs. The primary route of CAF demethylation in AS treatment was CAF, THB, 7-MX, and XAN, but the primary route of CAF demethylation in SAT was CAF, TEP, 1-MX, and XAN. Among transformation processes on profens, hydroxylation was important in the biotransformation of ibuprofen and fenoprofen as well as demethylation and hydrogenation in the biotransformation of naproxen and ketoprofen. For the transformation of profens, hydroxylation was expected in all cases except KTP in SBR, and demethylation was expected except KTP and FNP in SBR. These indicated that SAT obtained a wider range of hydroxylation and demethylation than AS treatment on biodegradation of profens. From the perspective of practical use, combination of SAT and AS treatment is suitable for wastewater reclamation.

In summary, sorption and biodegradation were the main removal mechanisms in SAT. SAT obtained stronger sorption including biological sorption, and has a wider range of biodegradation pathways than AS treatment, but AS treatment biodegrades micropollutants faster than SAT. From the perspective of characteristics of compounds, SAT shows strong sorption on the compounds having high K_{oc}, and effective biodegradation on the carboxylic compounds including halogenated acid. Therefore, cooperation of SAT and AS treatment was practical for wastewater reclamation to control micropollutants.

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Chapter 1 Introduction

1.1. Background

With fast developing urbanization, the problem of water scarcity became more and more serious around the world, and people have difficulty in accessing improved sanitation. Together with urbanization, overexploitation of water resource and discharge of pollutants lead to the further deterioration of water quality [1]. For example, wastewater water treatment plants (WWTPs) discharge a large amount of effluents every day, and pollutants in the effluents enter aquatic environments because of the limited capability of the wastewater treatment [2]. Wastewater reclamation has been considered as a feasible option to meet water demands and minimize the pollution in aquatic environment [3]. Wastewater after suitable treatment can be reused for many purposes such as industrial, agriculture, landscape use (non-potable use), indirect potable use and even direct potable use [4]. In fact, communities in arid areas have used the effluent from WWTPs as an alternative water resource [5-7]. United States, Europe, Israel and China had started reclamation projects [8-15]. Though most reclamation projects were for non-potable use, the reclamation plant in Windhoek, Namibia was the first reclamation plant using effluent for direct potable use since 1969 [16]. Furthermore, United States has started to employ the wastewater effluent for indirect potable use [17]. .

Though common pollutants such as NH_4^+-N, chemical oxygen demand (COD) and total phosphorus (TP) were effectively removed in WWTPs, micropollutants were still frequently detected in the effluent of WWTPs. This caused the environmental risk on ecosystem and could pose health risks to human. For improving water quality of wastewater effluent, advanced treatments such as advanced oxidation processes (AOPs), membrane technologies and soil aquifer treatment (SAT) are necessary after treatments in WWTP (i.e., activated sludge (AS) treatment). As an alternative choice to other advanced treatments for wastewater reclamation, in addition to the potential to improve the water quality, SAT has the advantages such as low operation cost, stable performance and the simplicity of its operation [18]. SAT is an option of artificial groundwater aquifer recharge, in which water was introduced into the groundwater through soil percolation under controlled conditions. During soil passage, water quality is improved by filtration, adsorption, chemical and biodegradation processes [19].

Usually, SAT is applied after AS treatment, but their differences and similarities are not fully understood. In order to access the validity of cooperation of SAT and AS treatment (application of SAT after AS treatment) for wastewater reclamation, the

removal mechanisms of micropollutants should be evaluated, especially for reclamation projects for potable use. Among micropollutants, pharmaceuticals and personal care products (PPCPs) are an ideal group of compounds as the indicators of micropollutants because of their variety of properties (i.e., soil organic carbon-water partitioning coefficient (K_{oc}), dissociation constant (pK_a) and varieties of functional groups). Furthermore, PPCPs in wastewater effluent could pose potential environmental risks [20]. In addition, the previous studies focused on the removal efficiencies of micropollutants, and micropollutants cannot be completely mineralized in AS treatment and SAT. Not only PPCPs, but also their metabolites, showed the endocrine disruption potential to humans and wildlife [21, 22]. In this study, the transformation pathway and transformation types of PPCPs in AS treatment and SAT were evaluated to understand the difference and similarity of the transformation of micropollutants in these two processes.

Generally, most treatment facilities are not designed to adequately remove micropollutants including PPCPs [23, 24], indicating PPCPs are not entirely removed in the wastewater treatment process and released into the aquifer environment from WWTPs as pollutants. Hijosa-Valsero et al. [25] and Kobayashi et al. [26] reported the existence of PPCPs in the influents of WWTPs, and PPCPs were partly removed and still detected in the effluent of WWTPs Because of relatively complex chemical structures of PPCPs and the limitation of AS treatment [27-29]. More than 80 PPCPs and their metabolites were detected in various aquatic environments even in drinking water [30-32]. Hanamoto et al. [33] found that 69 PPCPs existed in Yodo river, Japan. For the removals of PPCPs in AS treatment, acetaminophen, naproxen, ketoprofen, ibuprofen, atenolol, DEET, and chloramphenicol were removed effectively (removal efficiencies >70%), but crotamiton, carbamazepine and clofibric acid were not removed readily. To improve the removals of micropollutants in wastewater effluent, the advanced treatment after AS treatment is necessary as discussed above.

As well as AS treatment, SAT cannot degrade all micropollutants including PPCPs completely [34, 35]. For evaluating the removals of micropollutants in SAT, understanding the removal mechanisms of PPCPs in SAT is necessary with respect to the properties of PPCPs. In addition, by evaluating the difference of removal mechanisms (i.e., sorption and biodegradation) between AS treatment and SAT, the legitimacy of the cooperation of SAT and AS treatment could be well evaluated on basis of the cooperation of different removal mechanisms (i.e., sorption and biodegradation).

1.2. Problems identification and needs for research

The removals of PPCPs in AS treatment and SAT were reported in the previous studies, but few studies mentioned their removal mechanisms in SAT. Furthermore, no research evaluated the possibility of the cooperation of AS treatment and SAT. Thus, the problem in the previous studies and the needs in this study were illustrated as follows.

Firstly, our understanding on the relationship between the removals and the physical chemical properties of pollutants is still poor, and the roles of SAT as an

additional process to wastewater treatment remain to be unclear in the previous studies. This study addresses these unanswered questions by evaluating the removals of various PPCPs in SAT. More specifically, the roles of sorption and biodegradation in the removals of different types of PPCPs in SAT were evaluated, and their removal efficiencies of PPCPs in SAT and AS treatment were compared for evaluating the legitimacy of cooperation of SAT and AS treatment.

Secondly, though the removal efficiencies of several PPCPs in SAT were reported in the previous studies, the relationship between operating conditions of SAT and properties of PPCPs is not clear. More specifically, the effects of packing materials, hydraulic retention time (HRT), and saturated condition in SAT on the removals of micropollutants are not well understood. Thus, the removals of PPCPs in SAT under different operating conditions should be evaluated with respect to their properties.

Thirdly, biodegradable PPCPs such as caffeine and profens (e.g., ibuprofen and ketoprofen) can be degraded in both SAT and AS treatment. Caffeine has three methyl functional groups in its chemical structure, and profens possess a common functional group (2-arylpropionic acid). From the perspective of chemical structure, methyl and carboxyl functional groups widely exist in the chemical structures of micropollutants. However, few researches mentioned the occurrence of PPCPs transformation products (TPs), and their biodegradation pathway in SAT is still unknown. Thus, the information on transformation of caffeine and profens obtained in this study is expected to enhance our understanding on the transformation of micropollutants in SAT and AS treatment.

1.3. Research objectives

Based on the above discussion, the overarching research goal of this study was to investigate the removal mechanisms of PPCPs in SAT, and some specific objectives have been identified as follows:

1) To investigate the influence of sorption and biodegradation on the removals of PPCPs in SAT.

2) To investigate the difference of biodegradation between SAT and AS treatment.

3) To investigate the effects of packing materials, HRT and saturated condition in SAT on the removals of PPCPs.

4) To investigate the difference of biodegradation pathway of caffeine and profens between SAT and AS treatment.

5) To evaluate the occurrence of caffeine and profen TPs in SAT and AS treatment.

In addition, the cooperation of SAT and AS treatment would be clarified by comparing the removal mechanisms between AS treatment and SAT.

1.4. Outline of the thesis

This thesis consists of seven chapters. Figure 1.1 shows the research structure of this thesis.

In Chapter 1, the background of water reuse and the prospect of SAT are introduced as well as the characteristics of PPCPs and their risks. According to the problems in previous studies, the needs of this study are explained. Furthermore, the objectives and the structure of this study are presented.

Chapter 2 presents our current knowledge of PPCPs as indicators of micropollutants with respect to their properties, followed by the previous studies on the removal efficiencies of PPCPs in SAT and AS treatment. In addition, the formation pathway of TPs in metabolism and the occurrence of TPs in aquifer environment in previous studies are presented to provide information on TPs exploration.

In Chapter 3, firstly, the roles of sorption and biodegradation in SAT are evaluated by comparing the removal efficiencies of PPCPs in lab-scale columns under sterilized and non-sterilized conditions. Secondly, the sorption characteristics of PPCPs between autoclaved soil and soil in SAT were compared to understand the contribution of biomass on the sorption in SAT. Thirdly, the degradation rates of biodegradable PPCPs between soil batch-treatment experiment and sequencing batch reactor (SBR) were compared to understand the difference of biodegradation between SAT and AS treatment. Fourthly, the removal efficiencies of PPCPs in SAT and SBR are compared to understand the difference of the range of biodegradation between AS treatment and SAT. Furthermore, in this part, the feasibility of cooperation of SAT and AS treatment is evaluated on basis of SAT column experiment.

As discussed in Chapter 3, sorption and biodegradation contribute to the removals of PPCPs in SAT. The performance of SAT can be influenced by the operating conditions. In Chapter 4, the relationship between the operating conditions and the removal efficiencies of PPCPs is evaluated. Packing materials, HRT and saturated conditions are chosen as selected operating conditions in the column scale experiment. Combined with results in Chapter 3, the effects of operating conditions in SAT on the removals of micropollutants are discussed.

In Chapter 5, the difference in the demethylation of caffeine TPs between SBR and soil batch-treatment experiments is evaluated. In addition, ketoprofen, ibuprofen, fenoprofen and naproxen are chosen as target profens for evaluating the transformation types of carboxylic compounds in SAT and AS treatment. Overall, the possibility of cooperation of AS treatment and SAT is discussed not only from the perspective of the removal efficiencies of PPCPs, but also their transformation pathways.

Conclusions of this study are summarized in Chapters 6, followed by recommendations for further study.

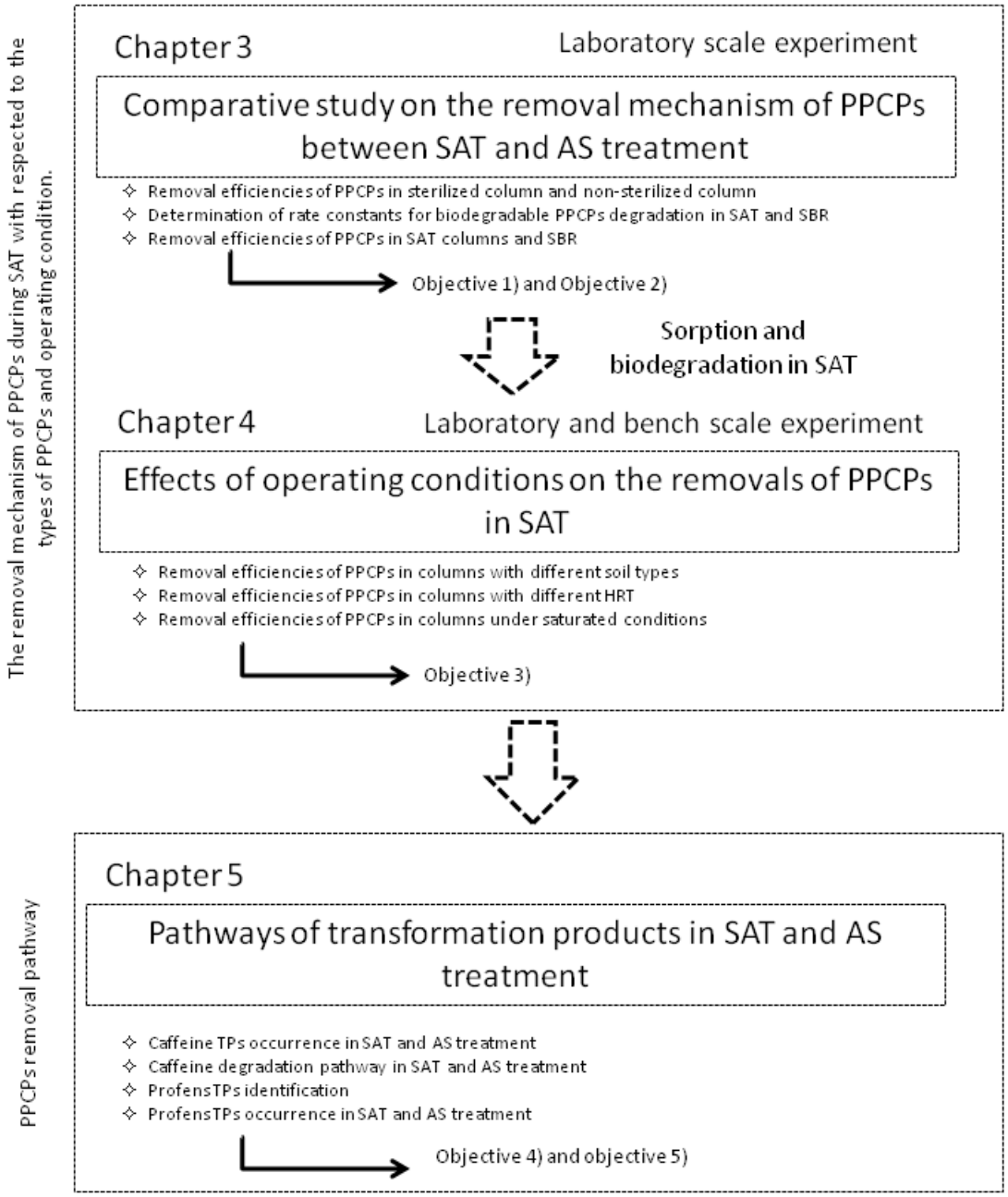

Figure 1.1 –Structure of this study.

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Chapter 2 Literature Review

2.1. Introduction

AS treatment is a traditional treatment in WWTPs. In AS treatment, most pollutants such as suspended solids (SS), COD, and NH_4-N can be effectively removed, but micropollutants are still detected in the effluents, and they enter into receiving waters through effluent discharge [1]. In addition, wastewater effluent was an alternative water resource to relieve the water crisis. For improving the water quality of wastewater effluent, SAT is an option for wastewater reclamation, and has the potential on removing micropollutants through physical, chemical and biological processes [2], and the performance of SAT on the removals of micropollutants is determined by operating conditions and site characteristics. The removal mechanisms and operating conditions in SAT will be introduced in this chapter.

Among micropollutants, PPCPs are an ideal group of compounds as indicators of micropollutants because of their varieties of properties. In this chapter, the categories and chemical structures of PPCPs are presented. SAT and AS treatment have the potential on the removals of micropollutants including PPCPs. In this chapter, the removal efficiencies of PPCPs in AS treatment and SAT were summarized according to the previous studies.

Caffeine and profens were chosen as parent compounds for the study of TPs because of their chemical structures and the occurrence in A_2O water. In this chapter, demethylation pathways of caffeine in the previous studies are summarized. In addition, the metabolites of profens in human and their occurrences in the water environment are presented.

2.2. SAT

SAT is an artificial groundwater aquifer recharge by introducing wastewater effluent into groundwater through soil percolation under controlled conditions. During the percolation process, the water quality of wastewater effluent is improved by physical, chemical and biological processes [2]. From the perspective of practical use, SAT contains unsaturated zone for natural filtration and saturated zone for storage in the treatment process. In addition, On basis of the water quality of wastewater effluent, land

availability and the usage of wastewater effluent, SAT can be complemented by various treatment technologies (i.e., ozonation and chlorination) for wastewater reclamation.

Generally, SAT are applied after the secondary treatment in WWTPs for wastewater reclamation. For example, Shafdan treatment plant (Dan region in Israel) is one of the biggest reclamation sites applying SAT for wastewater reclamation since the 1970s. The plant infiltrates a secondary effluent after nitrogen elimination at 130×10^6 m^3/year through a series of open ponds. The cycle lasts four days and the recharge rate is 70-210 m/year [3]. In addition, Shafdan plant has applied a short SAT combined with nanofiltration. This combination of technologies was found to be superior to the conventional SAT technology in terms of land use, treatment capacity, and water quality. Furthermore, this method enabled quick and efficient removals of microorganisms and micropollutants, resulting in the production of water near drinkable quality and suitable for irrigation use [4]. SAT was also applied in U.S and Europe such as Sweetwater recharge facilities in Tucson, Arizona in U.S [5], and SAT project located in Germany [6]. According to the previous studies, SAT showed significant removals on effluent organic matters (EfOM) [7] and micropollutants [8]. However, the performance of SAT is still affected by the quality of feeding water, site characteristics and operating conditions [9]. Usually, feeding water and site characteristics were decided by existing conditions such as the design capacity and the location of WWTPs, so the improvement of SAT should be started from the perspectives of optimization of operating conditions.

2.2.1. Removal mechanisms in SAT

In SAT, the removals of organic compounds, nitrogen, phosphorus, SS, bacteria and viruses are achieved by sorption, chemical reaction, biodegradation, die-off and predation[10-13]. For the sorption of organic compounds, many factors (i.e., the composition of water, the organic carbon content of soil, and the temperature) can influence the extent and rate of sorption to soil [14]. The major mechanisms of sorption have been proposed to involve ion exchange (electrostatic interaction), ligand exchange surface complexation, hydrophobic interaction, entropic effects, hydrogen bonding and cation bridging [15]. Besides sorption, biodegradation is also expected to contribute to the removals of organic pollutants in SAT. For example, heterotrophic bacteria can consume and oxidize organic substances to create new cell material and generate energy for growth and maintenance [16]. Furthermore, heterotrophic bacteria can use dissolved oxygen (DO) in SAT to biodegrade natural organic matter (NOM) [17].

2.2.2. Soil types

The performance on the removal of pollutants in SAT largely depends on the soil properties (composition and structure). Soil can be classified by the relative amounts of clay, silt and sand [18]. For the choice of SAT packing materials, Ho et al. [19] mentioned that not all soils are suitable for the removals of pollutants by SAT, and soils with large amount of clay fractions have to be avoided because of its impermeability. Higher infiltration capacity can be expected in sand, but pollutants were removed less effectively in sand than in soil. According to the works of Crites et al. [20] and Pescod et al. [21], the suitable surface soils for SAT systems are the fine sand, loamy sand and

sandy loam. In this study, weathered granite soil (WGS), Toyoura standard sand (TS), and standard sand were used, which are typical in West of Japan. Their characteristics will be presented in Part 3.2.2.

2.2.3. HRT

Usually, easily removable pollutants were removed in the top layer of SAT [22]. However, further removals of micropollutants are necessary for high water quality. Thus, for improving water quality of wastewater effluent, controlling HRT is important for the removals of micropollutants. For example, with increasing retention time during SAT, the aromatic carbon contents in SAT effluent decreased [23, 24]. Several guidelines for wastewater reclamation have mention that wastewater effluent from WWTPs should be treated in SAT with a certain HRT before reuse. For example, groundwater recharge regulations in California specify a minimum residence time of one year for injected water and six months for surface spreading [25]. During long-term operation of SAT, not only chemicals, but also bacteria and virus can be effectively removed by precipitation, adsorption, ion exchange, biological degradation, nitrification, denitrification, and inactivation [10]. Brissaud et al. [26] reported that HRT variation in the porous media column could influence the inactivation rate of bacteria in SAT.

2.2.4. Saturated condition

Saturated and unsaturated (vadose) zones in natural soil act as the media for physical, chemical and biological reactions [27], and these reactions substantially reduce the concentrations of organic compounds and inorganic compounds [28]. The vadose zone is characterized by the availability of oxygen, which is highly important in promoting aerobic biodegradation processes and nitrification [29]. In addition, aerobic condition could affect the redox condition in SAT, resulting in a significant influence on the kinetics of dissolved organic carbon (DOC) degradation [30]. In the saturated zone, a greater portion of the residence time occurs for the degradation of micropollutants, but DO for their biodegradation is limited [31].

2.2.5. Position of this research

Though the water quality of wastewater effluent through SAT is better than WWTPs effluents, its quality could be slightly different from native groundwater [28]. For example, micropollutants were frequently detected in SAT effluent. In addition, the removals of common pollutants (i.e., NH_4^+-N, DOC, and Total phosphorus (TP)) in SAT under different operating conditions were well understood in previous studies, but the removals of micropollutants in SAT under different conditions were not well understood. More specifically, the removal mechanisms of micropollutants in SAT are still not clear. Therefore, in this study, the contribution of biodegradation and sorption to the removals of PPCPs were evaluated.

2.3. PPCPs

This study is a part of a CREST project funded by JST, and 17 micropollutants were chosen as target compounds for safety evaluation in this project [32] (Table 2.1). According to the results in this project, the risks of micropollutants cannot be ignored because their concentrations in A_2O water were close to standards or guideline values [32] (Figure 2.1). As shown in Table 2.1, the K_{oc} values of organic micropollutants range from 4 (1,3-dichloro-2-propanol) to 30000 (E2), and the pK_a values of organic micropollutants range from 0.26 (EDTA) to 13.6 (chloroform). Most target compounds possess halogen, and six compounds have carboxyl groups. Similarly, PPCPs have varieties of physical-chemical properties such as K_{oc}, pK_a, and functional groups (Table 2.2), so PPCPs can be considered as indicators of micropollutants in SAT and AS treatment. The information on environmental risks of PPCPs is insufficient according to previous studies, but their occurrences and behaviors in wastewater effluent could cause ecological toxicity [33]. The concentrations of PPCPs were generally at trace levels (ng/L to μg/L) in the aquatic environment, and they can be sufficient to induce toxic effects because drug molecules are designed to interact with biological structures [34]. With increasing urbanization, the significance of PPCPs is likely to impose an increased risk on ecosystem and possible risk on human health [35]. Based on the concentrations and detection frequencies of PPCPs in the wastewater effluents in Japan [36, 37], target PPCPs in this study were selected as shown in Table 2.2. Their K_{oc} values range from 0.69 to 61000, and their pK_a values range from 1.4 to 9.5. The existences of carboxyl groups and halogens were indicated in Table 2.2. Selected PPCPs are expected to be indicators of micropollutants as they have similar ranges of K_{oc} and pK_a. Furthermore, the behaviors of carboxylic micropollutants and halide micropollutants in SAT could be evaluated based on the removals of selected PPCPs in this study. The environmental risk of the selected PPCPs would be discussed in 2.3.2.

Table 2.1 –Properties of organic micropollutants in the CREST project.

Name	K_{oc}	pK_a	Biodegradability	COOH	Halogen
Chloroform	34-196	13.6	Anaerobic	-	+
Bromodichloromethane	53-251	12.9	NO	-	+
Dibromochloromethane	84	12.3	anaerobic	-	+
Bromoform	116, 126	11.8	Denitrification Methanogenesis	-	+
Chloroacetic acid	31	2.87	Yes	+	+
Dichloroacetic acid	75	1.26	Weak	+	+
Trichloroacetic acid	130	0.51	Weak	+	+
NDMA	68-118		Weak	-	-
Dichloroacetronitrile	13		No	-	+
E2	30000	10.33	No	-	-
PFOA	26000	2.80	No	+	+
1,4-dioxane	29		Slow	+	-

EDTA	313	0.26	Weak	+	-
1,3-dichloro-2-propanol	4		Yes	-	+

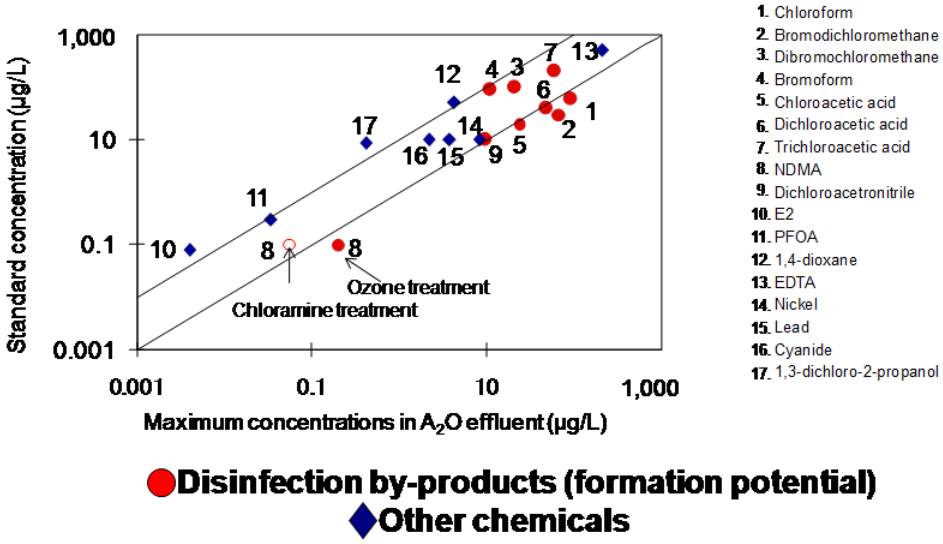

Figure 2.1 – The standard concentrations and the maximum concentration of micropollutants in A_2O effluent.

Table 2.2 –Properties of selected PPCPs.

Name	K_{oc}	pK_a	COOH	Halogen	Use/category	Abbr.
Ampicillin	6.423	2.5, 7.3	+		Antibiotic	AMP
Lincomycin	69	7.6			Antibiotic	LCM
Oxytetracycline	4.319	9.5			Antibiotic	OTC
Tetracycline	0.69	3.3			Antibiotic	TC
Azithromycin	3100	8.74			Antibiotic	AZM
Clarithromycin	150	8.99			Antibiotic	CAM
Ciprofloxacin	61000	6.09	+	+	Antibiotic	CPFX
Levofloxacin	44143	6.24, 8.74	+	+	Antibiotic	LVFX
Norfloxacin		6.34, 8.75	+	+	Antibiotic	NRFX
Enrofloxacin	15800	5, 8-9	+	+	Antibiotic	NRFX
Trimethoprim	75	7.12			Antibiotic	TRM
Sulfadimethoxine	285.2	6.91			Antibiotic	SDIME
Sulfamethazine	208	7.59			Antibiotic	SMT
Sulfamethoxazole	72	1.6, 5.7			Antibiotic	SMETH

Triclosan	15892	7.9		+	Antibiotic	TRC
Erythromycin	570	8.88			Antibiotic	ERY
Chlortetracycline	6.575	1.4		+	Antibiotic	CTC
Antipyrine	11.65	4.15			Analgesic	ATP
Diclofenac	245	4.5	+	+	Analgesic	DCF
Fenoprofen	233.8	4.15	+		Analgesic	FNP
Naproxen	330	4.45	+		Analgesic	NPX
Ketoprofen	229	4.2	+		Analgesic	KTP
Isopropylantipyrine	84.96	4.91			Analgesic	IPP
Mefenamic acid	880.1	3.49	+		Analgesic	MFA
Ibuprofen	3400	9.38	+		Analgesic	IBP
Aspirin	100		+		Analgesic	ASP
Acetaminophen	41	9.6			Analgesic	ACEAM
Crotamiton	244	10.45			Analgesic	CRT
Atenolol	4.081	3.83			Antiarrhythmic	ATL
Disopyramide	288	3.18			Antiarrhythmic	DSP
Bezafibrate	204	4.5	+	+	Antilipidemic	BZF
Clofibric acid	42.97	3.18	+	+	Antilipidemic	CFB
Gemfibrozil	430	4.5	+		Antilipidemic	GFB
DEET	300				Rejectant	DEET
Benzophenone	517				Ultraviolet absorber	BP
Caffeine	741				Cardiac	CAF
Carbamazepine	510	13.9			Antipilepsy	CBM
Primidone	23.84	11.5			Antipilepsy	PRM
Sulpiride	13.85	9.12			Peptic ulcer	SLP
Theophylline	517				Cardiac	TEP
Diltiazem	197.8	8.06			Blood-vessel dilator	DTZ
Furosemide	302	3.8,7.5	+	+	Blood-vessel dilator	FSM

2.3.1. Definition and classification of PPCPs

PPCPs are a group of various chemicals used for illness treatment and personal care, which consist of all drugs including the new genre of biologics, diagnostic agents, nutraceuticals, and other consumer chemicals such as fragrances and sunscreen agents. The number of registered pharmaceutical ingredients is over 3000, and the number of pharmaceuticals used to human is expected to increase continuously in the future [38]. After metabolism of pharmaceuticals in human body and cleansing of cosmetics, the original drugs and their metabolites in excrement are discharged into sewage. In addition, the effluent from hospitals is another important source of PPCPs. For example, in Germany, it is estimated that amounts of up to 16000 tons of pharmaceuticals are disposed from human medical care and 60~80% of those compounds are discharged into sewages each year [39, 40]. PPCPs are commonly detected in the influents of WWTPs, and cannot be removed completely through AS treatment. Thus, PPCPs will ultimately be present in wastewater effluent or enter the aquatic environment. The fate of PPCPs is shown in Figure 2.2.

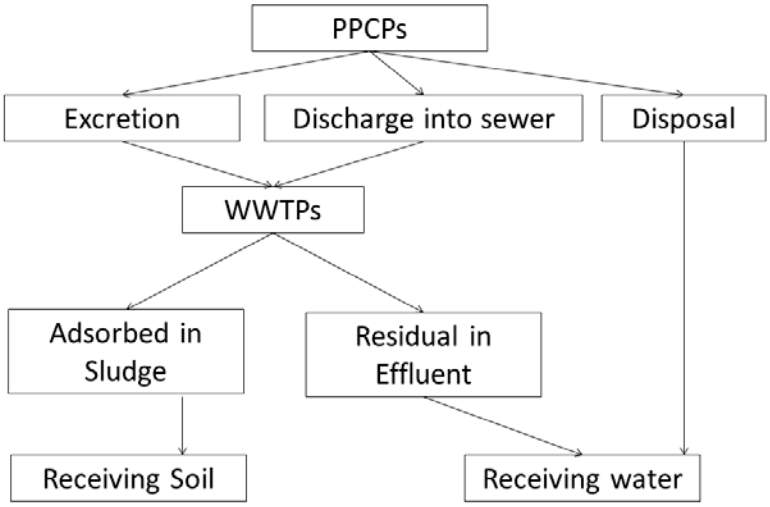

Figure 2.2 – The pathway of PPCPs.

PPCPs are a highly diverse group of compounds with different chemical structures as well as biological and physiochemical properties, resulting in varieties of degrees of removal in treatment processes. Suárez et al.[41] reported that pK_a and K_{ow} influenced the removals of PPCPs in activated sludge treatment. Furthermore, Delgado et al.[42] proposed the potential of quantitative structure-activity relationship (QSAR) like models to rank treatability of PPCPs, including parent compounds, metabolites and TPs in drinking water treatment. PPCPs with similar structures could perform similarly in treatment processes. However, the relationship between removal mechanisms in SAT and properties of micropollutants is not clear. In this study, PPCPs are classified according to their medical purposes (e.g., antibiotic, analgesic, antilipdemic, antiarrhythmic, blood-vessel dilator and so on), and the removals of PPCPs in SAT were evaluated with respect to physical-chemical properties and their chemical structures.

2.3.1.1. Antibiotics

Antibiotics are a type of medicines used against bacteria, and are often used in the medical treatment of bacterial infections. They may either kill bacteria or inhibit the growth of bacteria. The presence of antibiotics in water environment not only can cause changes in the microbial communities [43], but also can develop the antibiotic resistance of pathogenic bacteria [44, 45]. In effluents of WWTPs, antibiotics were widely detected according to previous studies [46, 47] (Table 2.3). In this study, AMP, LCM, AZM, CAM, ERM, TRM, OTC, TC, CTC, CPFX, LVFX, NRFX, ERFX, TRC, SDIME, SMT, and SMETH were chosen as target antibiotics, and their chemical structures were shown in Figure 2.3. Among selected antibiotics, CPFX, LVFX, NRFX, and ERFX are fluoroquinolones with high K_{oc} values. The values of pK_a of LCM, AZM, CAM, and ERM range from 7.60 to 8.99, in about the same range as the pH value in aquifer environment or slightly higher. Thus, by evaluating their removals in SAT, the influences of the K_{oc} and pK_a values on the removals of micropollutants in SAT were evaluated.

Table 2.3 –Occurrences of antibiotics in the influents and effluents of WWTPs.

Compounds	Concentration in the influent (ng/L)	Concentration in the effluent (ng/L)
Amoxicillin	N.D. (<59) [46]	N.D. (<5.9) [46]
Ampicillin	N.D. (<60)-46300 [46]	N.D. (<5.7)-109 [46]
Azithromycin	N.Q. (<410)-1350 [46]	5.03-818 [46]
Benzylpenicillin	N.D. (<10)-55.8 [46]	N.D. (<3.6)-N.Q. (<17) [46]
Ceftiofur	N.D. (<46)-N.Q. (<150) [46]	N.D. (<6.3) [46]
Chloramphenicol	N.Q. (<12)-33.7 [46]	N.D. (<1.8)-13.4 [46]
Chlortetracycline	N.D. (<57) [46]	N.D. (<5.7) [46]
Ciprofloxacin	N.Q. (<320)-195 [46]; 2789 [48]	N.Q. (<1.5)-50.3 [46]; 257[48]
Clarithromycin	394-14800 [46]; 136 [48]	68.5-5100 [46]; 66[48]
Enrofloxacin	N.D. (<74)-23.5 [46]	N.Q. (<74)-23.5 [46]
Erythromycin	N.Q. (<54)-1870 [46]; 143 [48]	55.5-458 [46]; 72[48]
Levofloxacin	205-3960 [46]; 1178 [48]	56.1-152 [46]; 105 [48]
Lincomycin	N.D. (<20)-40.5 [46]; 636	N.D. (<2.0)-24.8 [46]
Nicarbazin	N.D. (<110)-10.3 [46]	N.D. (<1.0)-N.Q. (<3.5) [46]
Norfloxacin	N.Q. (<190)-468 [46]	N.D. (<5.6)-68.0 [46]
Novobiocin	N.D. (<2.2)-134 [46]	N.D. (<1.1)-61.1 [46]
Oxytetracycline	N.D. (<2.0)-6.77 [46]	N.D. (<4.4)-9.28 [46]
Roxithromycin	28.4-209 [46]	8.54-130 [46]
Salinomycin	N.D. (<4.4)-60.2 [46]	N.D. (<2.7)-N.Q. (<7.4) [46]
Sulfadimethoxine	N.D. (<100)-71.0 [46]	N.D. (<10)-18.8 [46]
Sulfadimidine	N.D. (<33)-15.0 [46]	N.D. (<3.3)-9.36 [46]
Sulfamerazine	N.D. (<2.0)-15.1 [46]	N.D. (<3.5)-8.28 [46]
Sulfamethoxazole	N.D. (<61)-184 [46]; 2007 [48]	N.Q. (<20)-153 [46]; 342 [48]
Sulfamonomethoxine	N.D. (<4.9)-19.4 [46]	N.D. (<2.5)-N.Q.(<8.2) [46]
Tetracycline	N.D. (<36)-89.0 [46]	N.D. (<36)-89.0 [46]
Tiamulin	N.D. (<9.8)-47.2 [46]	N.D. (<0.98)-5.41 [46]
Triclosan	184-327 [46]; 511 ±243 [49]; 128 [48]	N.Q. (<8.0)-47.3 [46]; 26-330 [49] 15 [48]
Trimethoprim	N.D. (<59)-111 [46]; 992 [48]; 373 [50]; 80 [51]; 1510-4670[52]	N.Q. (<20)-52.5 [46]; 149 [48]; 390 [50]; 40 [51]; 380-1200 [52];
Tylosin	N.D. (<52)-1.02 [46]	N.D. (<5.2)-0.631 [46]

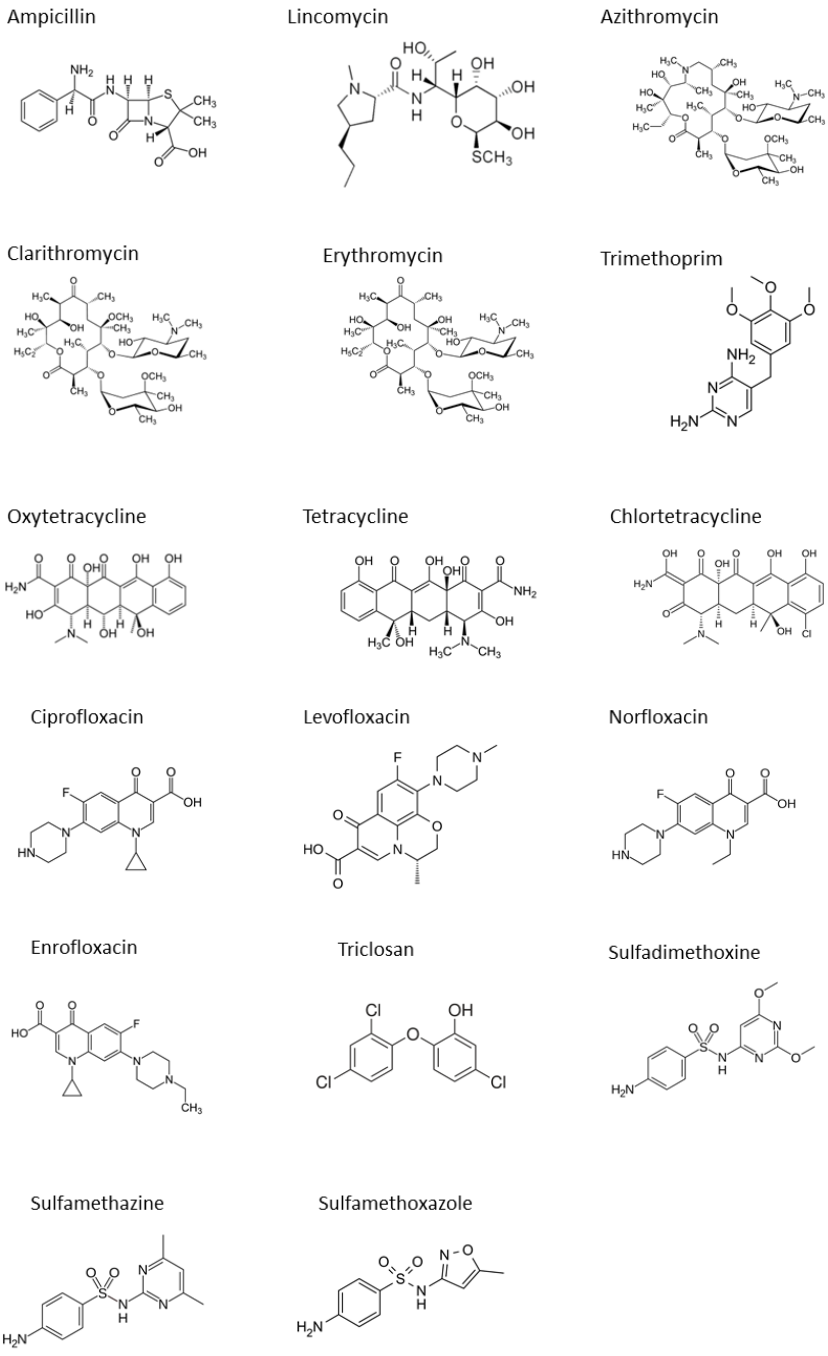

Figure 2.3 –The chemical structures of target antibiotics.

2.3.1.2. Analgesics

Analgesics are a group of drugs used for analgesia (pain reliever) in the peripheral and central nervous systems. Among analgesics, non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the world. NSAIDs are used by 30 million people every day, and their consumption in the U.S., United Kingdom, Japan, France, Italy, and Spain had

increased largely each year with a market of 11.6 billion dollar in 2008 [53]. In the U.S., around 43 million adults took ASP at least three times per week for more than 3 months (i.e., regular users), and more than 29 million adults were regular users of NSAIDs in 2010, an overall increase of 57% in ASP use and 41% in NSAID use compared with 2005 [54]. From the perspective of chemical structure, NSAIDs consist of an acidic moiety (i.e., carboxylic acid and enols) attached to a planar aromatic functionality [53]. Carboxyl groups widely are present in the chemical structure of micropollutants such as haloacetic acid. Among selected analgesics, propionic acids (i.e., KTP, NPX, IBP, and FNP) and acetic acids (i.e., DCF) possess carboxyl groups. Analgesics were widely detected in the influents and effluents of WWTPs in previous studies [46, 47], and their occurrences in WWTPs are shown in Table 2.4. The existence of analgesics can cause adverse effects on ecosystem. For example, the decline of the population of vultures in Pakistan because of the exposure of diclofenac [55]. The mixture of several analgesics can induce more complex toxicity to living organisms [56]. For example, combined occurrence of DCF, IBP, NPX, and acetylsalicylic acid in water demonstrated synergistic adverse effect on Daphnia [57]. In this study, ATP, IPP, DCF, FNP, NPX, KTP, IBP, MFA, ASP, ACEAM, and CRT were chosen as target analgesics referring to the occurrence of analgesics in previous studies [58] (Figure 2.4).

Table 2.4 –Occurrences of analgesics in the influents and effluents of WWTPs.

Compounds	Concentration in the influent (ng/L)	Concentration in the effluent (ng/L)
Acetaminophen	85.1-15900 [46]; 37690 [48]	N.Q. (<4.2)-21.9 [46]; N.D [48]
Antipyrine	N.D. (<50)-14.0 [46]	N.D. (<5.0)-11.2 [46]
Diclofenac	107-151 [46]; 67 [48]; 426 [50]; 160 [51]; 60-1160 [52]	74.6-141 [46]; 21 [48]; 345 [50] 120 [51]; 6-500 [52]
Ethenzamide	N.D. (<26)-30.5 [46]	N.Q. (<8.8)-17.9 [46]
Fenoprofen	N.D. (<5.6)-N.Q. (<19) [46]; 9.48-80.6 [49]	N.D. (<2.8)-8.41 [46]; 1.59-9.22 [49]
Ibuprofen	205-3960 [46]; 381-1130 [49]; 14333 [48]	56.1-152 [46]; 1.41-177 [49]; 40 [48]
Isopropylantipyrine	N.D. (<20)-40.5 [46]	N.D. (<2.0)-24.8 [46]
Ketoprofen	N.D. (<110)-10.3 [46]; 108-369 [49]	N.D. (<1.0)-N.Q. (<3.5) [46]; 68.1-219 [49]
Mefenamic acid	N.Q. (<190)-468 [46]; N.D. [49]	N.D. (<5.6)-68.0 [46]; N.D. [49]
Naproxen	N.D. (<2.2)-134 [46] 38.0-230 [49]; 11912[48]	N.D. (<1.1)-61.1 [46] ; 12.0-139 [49];53 [48]

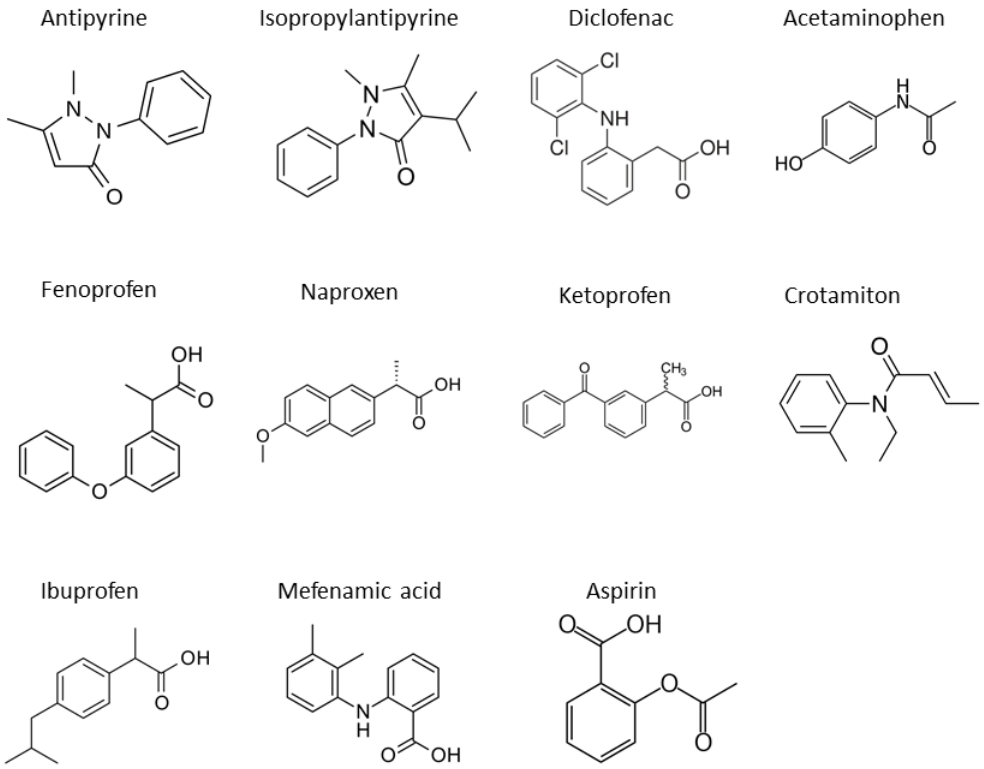

Figure 2.4 –The chemical structures of target analgesics.

2.3.1.3. Other PPCPs

Besides antibiotics and analgesics, some other PPCPs also widely existed in the influents and the effluents of WWTPs. In this study, ATL, DSP, BZF, CFB, GFB, DEET, BP, CAF, CBM, PRM, SLP, TEP, DTZ, and FSM were chosen, and their chemical structures are shown in Figure 2.5. Among these PPCPs, ATL and DSP are antiarrhythmics, which are frequently detected in the aquifer environment [59, 60]. BZF, CFB, and GFB belong to antilipidemics, possessing carboxyl groups in their chemical structures. CBM and PRM possess amide functional groups. These compounds are ideal to study the influence of functional groups on the biodegradation in SAT.

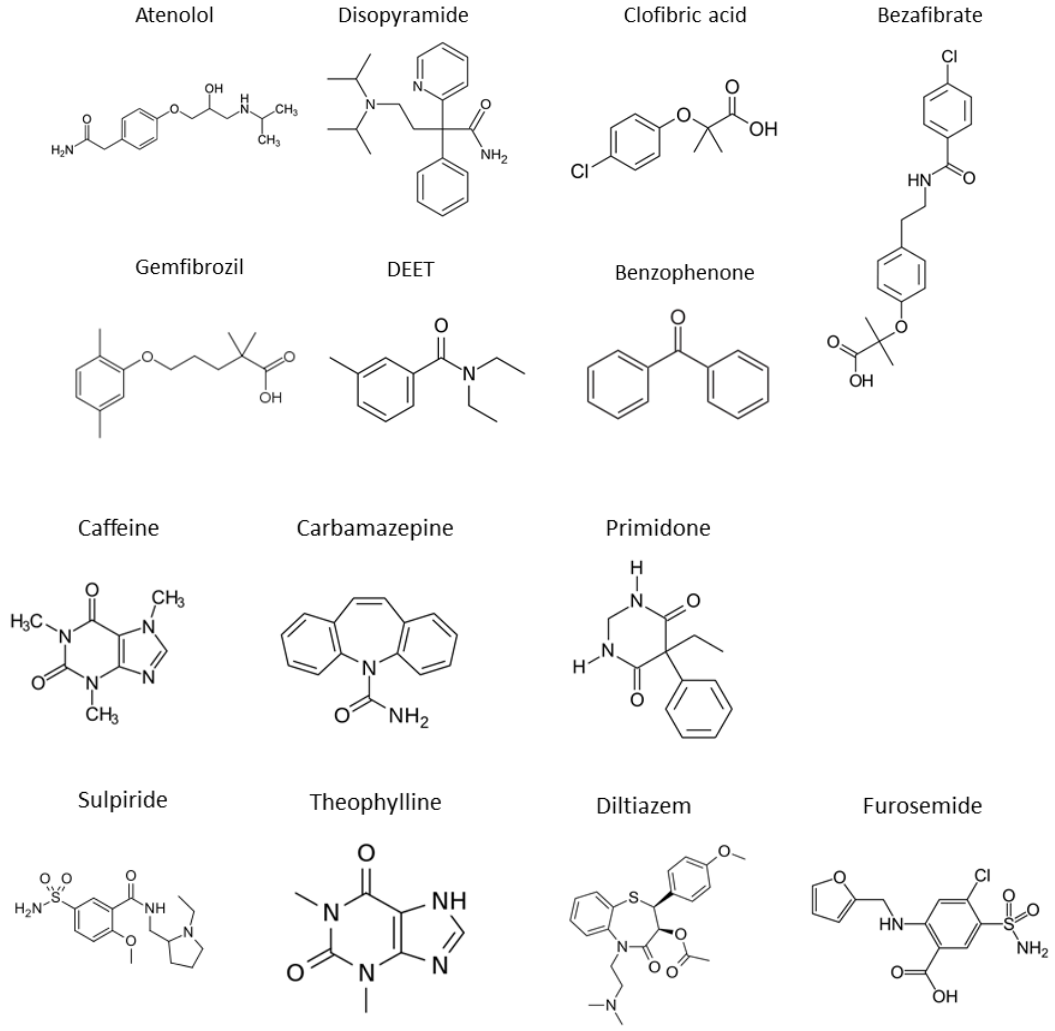

Figure 2.5 –The chemical structures of other PPCPs.

2.3.2. Environmental risk of PPCPs

Besides being indicators of micropollutants, PPCPs themselves may pose an environmental risk because they have been designed to have a physiological effect on humans and animals [56]. Actually, however, the side effects of PPCPs to human upon consumption of drinking water are negligible, and PPCPs do not showed an appreciable risk to human health from potential environmental exposure [61-63]. The acceptable daily intakes (ADI) and predicted no effect concentrations (PNEC) for children ranged from 4.2 (TRM) to 340 (ACEAM) μg/(kg・day) and from 6.0×10^{4} (TRM) to 4.9×10^{6} (ACEAM), respectively [61], which higher than their concentrations in the water environment.

Though the information on the health risk of PPCPs on human is insufficient, eco-toxicological impacts of CBM, CFB, and DCF in treated wastewater were investigated by using bacteria, algae, invertebrates and fish. CBM was found to be the most dangerous

compound for the aquatic environments [64]. Aris et al. [65] mentioned that high risk and acute toxicity of DCF were expected in Mekong River and Langat River. Furthermore, effects in aquatic organisms exposed to individual pharmaceuticals in the laboratory have been investigated regarding the environmental impacts of PPCPs by Galus et al. [66], and they showed that the chronic exposure of pharmaceutical mixtures in wastewater to fish leads to the histopathological changes of reproductive system.

In addition, environmental risk assessment (ERA) is used for evaluating possible acute or chronic aquatic toxicities of PPCPs. The ratio of predicted environmental concentrations (PEC) to PNEC of PPCPs in environment was used to evaluate their ecological toxicities in environment. PNEC values of target PPCPs in this study are shown in Table 2.5. For example, Stuer-Lauridsen et al. [67] mentioned that the ratios of PEC to PNEC for IBP and paracetamol were 1.8 and 7.1 in environmental samples of Denmark. That is, for some PPCPs, ecological toxicities are expected.

Table 2.5 –PNEC values of target PPCPs for ecological risk.

Name	PNEC(ng/L)	Name	PNEC(ng/L)
LVFX	510 [68]	TRM	6250[69]
IPP	15600[69]	ATP	100000 [75]
BP	1320[70]	SDIME	6300 [69]
ATL	48000[71], 630 [69]	DTZ	4500[76], 6300 [69]
LCM	10[72], 78[69]	TEP	500000[73]
CBM	5210[73], 63000[69]	CAM	52[73]
CRT	21000[73], 63000 [69]	DEET	5200[73], 500000 [69]
SLP	100000[73], 130000 [69]	CAF	5200[73], 182000[77]
AZM	50[73], 160 [69]	PRM	100000[73], 130000 [69]
DSM	63000[73]	IBP	2 [68], 9100 [67]
ACEAM	1400[73]	GFB	10000[78]
FSM	83000[73]	SMETH	900[73], 1600 [69]
FNP	20830[73]	MFA	20830[73], 50000 [69]
BZF	10000[73]	CFB	6400[79], 250000 [69]
DCF	10000[73], 63000 [69]	TRC	80[73]
KTP	160 [69], 15600 [74]	NPX	5200[73], 63000 [69]

2.3.3. Occurrences of PPCPs in WWTPs

A large number of PPCPs are detected in the influents of WWTPs because of their frequent use by human as presented above. In wastewater treatment, PPCPs such as antibiotics are resistant [80-82]. In addition, several PPCPs such as DCF and IBP are conjugated to polar molecules, and these conjugates can easily be cleaved during WWTPs to form their parent compounds again [80, 83]. Thus, the existences of PPCPs in wastewater effluents cannot be ignored.

In Japan, as Okuda et al. [84] reported, 17 of 33 PPCPs were detected at relatively high concentration (>100 ng/L) in the influents of WWTPs including SLP and DSP

(above 200 ng/L). The behaviors of PPCPs during different activated sludge systems (i.e., AS treatment, membrane bioreactors (MBRs), and SBRs) were evaluated in previous studies: Yang et al. [85] reported the occurrence of 19 PPCPs after various stages of treatment in an advanced wastewater reclamation plant monthly for 12 months, and they found that the concentrations of CAF, ACEAM, IBP, DEET, TC, and 17α-ethynylestradiol (EE2) had decreased by more than 90%, but ERM and CBM were resistant to biological treatments. Okuda et al. [36] reported that 56 PPCPs were detected in the primary sludge and 61 compounds were detected in the excess sludge of WWTPs.

Not only biodegradation, but also other mechanisms such as sorption can contribute to the removal of PPCPs in activated sludge system [47, 58]. The removals of selected PPCPs in WWTPs were summarized in Table 2.6. However, the removal efficiencies of PPCPs varied from WWTP to WWTP. For example, the removal efficiencies of CAM ranged from -45 to 54%. These indicated the limitations and uncertainty of activated sludge treatments on the removals of micropollutants including PPCPs, resulting in possible risks on the ecology in water environment as discussed above. Thus, for improving water quality in wastewater effluent, additional treatments such as SAT should be added after the secondary treatment.

Table 2.6 –Removals of PPCPs in WWTPs.

Compounds	*Removal efficiencies (%)*	*Treatment scale*
AMP	16.4, 42.1[86]	WWTP, full scale
	67.8, ND[86]	Batch
LCM	0[87]	WWTP, full scale
	69.4[88]	Batch
TC	86.4, 85.1, 78.4[89]	SBR, lab scale
AZM	0.4, 0.5[90]	Batch
CAM	0[87], -45, 14, 20[91], 54[92]	WWTP, full scale
CPFX	60, 63[87], 84, 86, 79, 96[93]	WWTP, full scale
NRFX	66[94]	WWTP, full scale
TRM	<1[95], 49[51], 69[96], 74 [91]	WWTP, full scale
	23.8[97]	Batch
SMETH	-250[51], 9, 29[91], 62[98], >98[99]	WWTP, full scale
	100[100]	SBR, lab scale
ERM	0[87], 23.8[101], 25[96], 26[94]	WWTP, full scale
	9.1, 4.5[102], 67.3[101]	MBR, pilot scale
DCF	22[51], 40-60[103], 69[104], 69[105]	WWTP, full scale
	58[106], 44-85[107], -8, 39[104]	MBR, pilot and lab scale
FNP	91.8-97.5[49]	WWTP, full scale
NPX	93[51], 55[108], 56.2[109], 15[110]	WWTP, full scale
	36, 41[111], 99.3[101]	MBR, pilot and lab scale
KTP	8[112], 51-100[113], 77[114], 92[93]	WWTP, full scale
	91.1, 89.6[115]	WWTP, pilot scale
MFA	91.54[1], 29.4[101], 50, 2[112]	WWTP, full scale
	74.8[101]	MBR, lab scale
IBP	20[112], 38, 93[87], 96[51], 97[106], 96-99.9[49]	WWTP, full scale
	80-100[116], 90.8[115]	WWTP, pilot scale
ASP	99.3-99.6[49], 81[105]	WWTP, full scale

ACEAM	99[117], 91.93[1], 98.4[101]	WWTP, full scale
	>99[111], 99.6[101]	MBR, pilot and lab scale
CRT	-5-24[49]	WWTP, full scale
ATL	10, 55[87], 79[118], 84[96]	WWTP, full scale
	65.5[101]	MBR, lab scale
BZF	15, 87[87], >95, 90[119], 80-100[103]	WWTP, full scale
CFB	26[106], 34[110], 51[105], 52[96], ND[112]	WWTP, full scale
GFB	16, 46[110], 69[105], 75[51]	WWTP, full scale
	89.6[101]	MBR, lab scale
DEET	19.2-46.2[49]	WWTP, full scale
CAF	85[117], 94[51], 99.7[96]	WWTP, full scale
	>98, 99[111]	MBR, pilot scale
CBM	<20[103], 14[104], -3, -43[120], -121, -193[93]	WWTP, full scale
FSM	8, 54[87]	WWTP, full scale

2.3.4. The removals of PPCPs in SAT

In SAT, biological, chemical, and physical processes occurring in soil can be expected to remove PPCPs when water go through soil, and their removals during SAT has been assessed in both field and lab-scale in previous studies. For example, IBP was readily degradable, but CBM and SMETH were more persistent in SAT [80, 121-123]. These suggested that the removals of PPCPs varied with the properties of PPCPs. In addition, the operating conditions also affected the removals of PPCPs in SAT: Degradation of EDCs and some PPCPs in soil were affected by environmental conditions such as temperature, pH, moisture content, organic carbon, the presence of specific microorganisms, and DO conditions [123-125]; Grunheid et al. [30] found that bank filtration system removed SMETH by 95%, but artificial recharge system removed only 53%. Onesios et al. [126] reported the removals of PPCPs during laboratory SAT simulation, and found that biphenylol, *p*-chloro-*m*-cresol, chloroprene, 5-fluorouracil, GFB, IBP, KTP, NPX, TRC, and valproic acid were effectively removed in laboratory columns. Drewes et al. [127] found that CAF, and inflammatory and lipid-regulating drugs were removed efficiently, but antiepileptic drugs (CBM and PRM) were persistent in ground water recharge. Referring to the works of Onesios et al. [58], the removals of PPCPs in subsurface flow and constructed wetlands, which are another managed aquifer recharge, were summarized in Table 2.7. As discussed above, both AS treatment and SAT have the potential on the removals of micropollutants. However, in the previous studies, the number of target PPCPs is limited, and the relationship of the removals of PPCPs and the properties of PPCPs was not clear. Therefore, the comparative study between AS and SAT based on removal mechanisms is necessary to evaluate the feasibility of cooperation of SAT and AS treatment.

Table 2.7 –Removals of PPCPs in soil treatments.

Compounds	Removal efficiencies (%)	Treatment scale
CAM	>88[96]	Subsurface flow
TRM	>92[96]	Subsurface flow
SMETH	82[96], 95, 53[30]	Subsurface flow

TRC	67.9[128]	Constructed wetlands, pilot scale
ERM	>95[96]	Subsurface flow
DCF	96, 73[129]	Constructed wetlands
	>87[130], 45, 0[131], >98[96]	Subsurface flow
FNP	>71[130]	Subsurface flow
NPX	92, 52[129]	Constructed wetlands
	>99[130], 90, 85, 80, 0, 47[131]	Subsurface flow
KTP	99, 97[129]	Constructed wetlands
	>77[130], 69, 0, 45[131]	Subsurface flow
IBP	96, 95[129]	Constructed wetlands
	>99[130], 80, 71, 62, 52, 17[131]	Subsurface flow, field scale
ATL	>93[96]	Subsurface flow
CFB	48.3[132]	Lab columns
GFB	>99[130]	Subsurface flow
CAF	98, 94, 99, 94, 85[131], >88[96]	Subsurface flow
CBM	<-4450[130], ND[133], 73[134]	Subsurface flow, field scale
	5[129]	Subsurface flow, lab scale
PRM	<-5[130]	Subsurface flow, full scale

2.4. Transformation products (TPs)

Toxicological properties, environmental occurrence, and removal efficiencies of PPCPs in WWTPs were reported in previous studies, but few studies were available on their possible TPs. PPCPs were transformed into metabolites, which were excreted in urine and feces with their parent compounds. Usually the metabolites were classified as Phase I and Phase II metabolites. Phase I metabolites are formed in *vivo* biochemical oxidation, reduction, and hydrolysis reactions [135]. Phase II metabolites (conjugated metabolites) are formed by the biochemical addition of a molecule such as glucuronic acid to the parent compound [136]. Some phase II metabolites were deconjugated back to their parent compounds again in wastewater treatments [137]. In this part, TPs of PPCPs in the previous studies were presented to provide information on the formation of TPs.

Transformation of pharmaceuticals in human has been evaluated extensively, but the degradation of pharmaceuticals in SAT had rarely been investigated. Referring to the knowledge on the metabolism in human body, degradation of pharmaceuticals such as hydroxylation and conjugation may contribute to the transformation of PPCPs in AS treatment and SAT: Miao et al. [138] and Yonetani [37] reported that hydroxylated metabolites of CBM occurred with higher concentration in wastewater than CBM. Biotransformation products in the previous studies were summarized in Table 2.8 referring to the work of Michael et al.[139]. For transformation pathway, to my best knowledge, no literature mentioned TPs and their formation pathways in SAT. Furthermore, virtually no data on the occurrences of TPs in Japan was reported. As discussed in Table 2.8, pharmaceuticals were biologically transformed under wastewater treatment conditions (mainly activated sludge treatment), but the specific routes and mechanisms were not clear. In this study, the transformation pathways of micropollutants in SAT were evaluated based on the transformation of PPCPs in SAT.

Table 2.8 –TPs of PPCPs detected in WWTPs and lab-scale biodegradation experiments.

Parent compounds	Biotransformation products	Biodegradation experiment
ATL	Atenololic acid[140]	Biodegradation experiment
BZF	4-chlorobenzoic acid[81]	Membrane bioreactor
CBM	10, 11-dihydro-10, 11-dihydroxycarbamazepine; 2-hydroxycarbamazepine [138]	Conventional wastewater treatment
CPFX	succinyl ciprofloxacin [141]	Membrane bioreactor
	N-acetylciprofloxacin [142]	Batch experiments with isolated *E. coli* strain
DCF	p-benzoquinone imine [143]	Fixed bed bioreactor under aerobic conditions
IBP	Hydroxyl-IBP, Carboxyl-IBP[144]	Bench scale biofilm reactors and in vitro batch
KTP	3-(hydroxy-carboxyl-methyl) hydratropic acid[81]	In vitro batch experiment with activated sludge
NPX	Desmethyl-NPX[81]	Membrane bioreactor
NRFX	succinylnorfloxacin[145]	Membrane bioreactor
SMETH	Hydroxyl-N-(5-methyl-1,2-oxazol-3-y)benzene-1-sulfonamide[146]	Batch experiment with *Rhodococcus rhodochrous*
TRM	Hydroxylated TRM[147]	In *vitro* batch experiment with vitrifying activated sludge

2.4.1. Identification of TPs

Generally, identification of TPs has been the greatest challenge, especially in environmental samples because of their low concentration and the complexity of sample matrix. Another analytical challenge related to the identification of TPs in environmental samples is the lack of standard compounds. Thus, analytical approaches for TP determination can be generally classified into three categories: target analysis (with reference standards), suspect screening (with suspected substances based on possible transformation information but no reference standards), and non-target screening (no transformation information, no reference standards) [148-150]. For the first approach, the occurrence of TPs is confirmed by a quantitative method using standard compounds. For the second approach, specific information for possible TPs (accurate mass spectra of both molecular and fragment ions related to possible TPs) was expected. For the third approach, it is difficult to get useful information by screening unknown compounds without any information on TPs. As Evgenidou et al. [135] suggested, a comprehensive target screening for TPs is necessary before unknown screening. In this study, the transformation of CAF was evaluated by target analysis and the transformations of profens were evaluated by suspect screening.

2.4.1.1. CAF TPs

CAF is a common purine alkaloid in most popular drinks especially tea and coffee, which is responsible for the stimulant action. CAF was also a cardiotonic widely used nonprescription human drug in the world [151, 152]. After consumed by humans, CAF

and its metabolites were discharged into wastewater treatment systems. CAF has been detected in many surface streams and the effluents of WWTPs according to previous studies [153-155]. From the perspective of chemical structure, CAF is a xanthine derivative, possessing three methyl groups in its chemical structure. Thus, CAF is an ideal compound for evaluating the demethylation of micropollutants.

Transformation of CAF and other xanthine derivatives in human body and other mammals has been studied extensively. The transformation of CAF consists of two pathways: Firstly, theophylline, paraxanthine and theobromine were formed by oxidative demethylation; Secondly, through C-8 hydroxylation, 1,3,7-trimethyluric acid and 6-amino-5[N-formylmethylamino] 1,3-dimethyl uracil were formed [156]. In the sequential demethylation, 1-methylxanthine, 3-methylxanthine, 7-methylxanthine and xanthine were formed by demethylase enzymes (N-1 demethylase, N-7 demethylase and N-3 demethylase)[157]. Overall, the demethylation pathway of CAF is shown in Figure 2.6. However, the demethylation pathway of CAF in SAT was still not clear, and no research compared the demethylation pathway of CAF in SAT with that in AS treatment.

In summary, because CAF possesses methyl functional groups in different position, the demethylation of CAF could be a good example to evaluate the demethylation process of micropollutants in SAT and AS treatment.

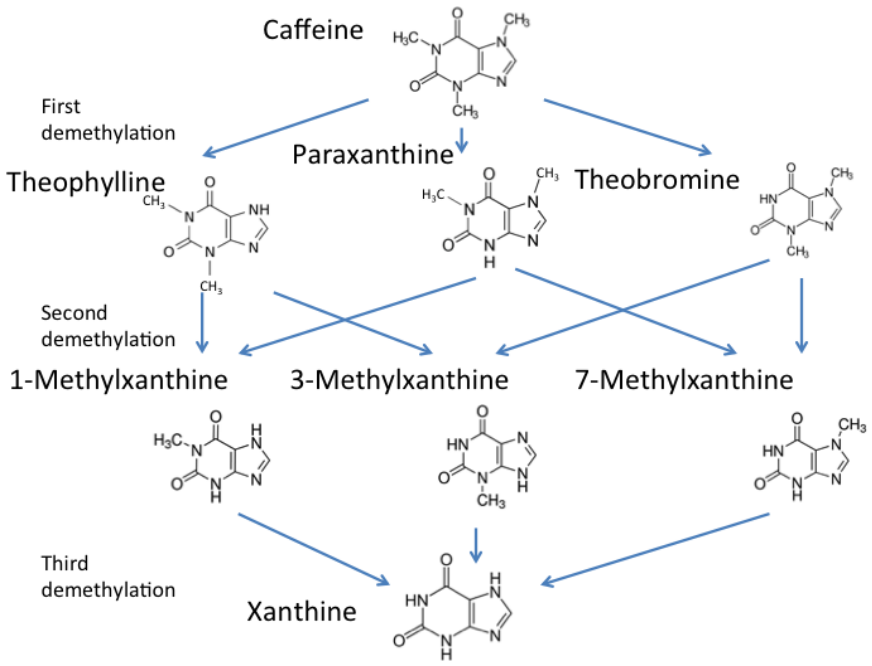

Figure 2.6 –The demethylation pathway of CAF in previous studies.

2.4.1.2. Profen TPs

2-arylpropionic acid (profens) is a class of compounds including widely used NSAIDs such as IBP, KTP, NPX, and FNP, which have anti-inflammatory and analgesic activities because of their ability to inhibit cyclooxygenase enzymes promoting

inflammation [158]. The consumption of IBP were several hundred tons per year in Germany and England [159]. Besides biodegradable PPCPs in this study, micropollutants such as chloroacetic acid, dichloroacetic acid, trichloroacetic acid, PFOA, and EDTA possess carboxyl groups. Through the biodegradation types of profens (carboxyl acids), the transformations of carboxylic compounds in SAT and AS treatment are evaluated. With the huge consumption, profens were detected in the influents and effluents of WWTPs according to the previous works [112, 160, 161]: Stuer-Lauridsen et al. [67] mentioned that PEC of IBP is 8.90 μg/L, and IBP was detected at 205-3960 ng/L in the effluents, which is close to PEC of IBP.

Transformations of profens in human were extensively studied in previous studies. After oral administration of IBP, hydroxyl-IBP and carboxyl-IBP were found as metabolites of IBP in urine (Figure 2.7) [162-164]. KTP was excreted in urine mainly with the conjugation with D-glucuronic acid [165]. In addition, hydroxylation of the aromatic ring and reduction of ketone functional group were expected in the biotransformation of KTP [166-169]. For the risk of profen TPs, Méndez-Arriaga et al. [170] mentioned that the hydroxylated derivatives of IBP is more toxic than their parent compound (IBP) by increasing the inhibition percentage of bioluminescence from *V. fischeri*.

In summary, profens contains arylpropionic acid as a common structure, and they can be considered as indicators for the transformation of carboxylic compounds.

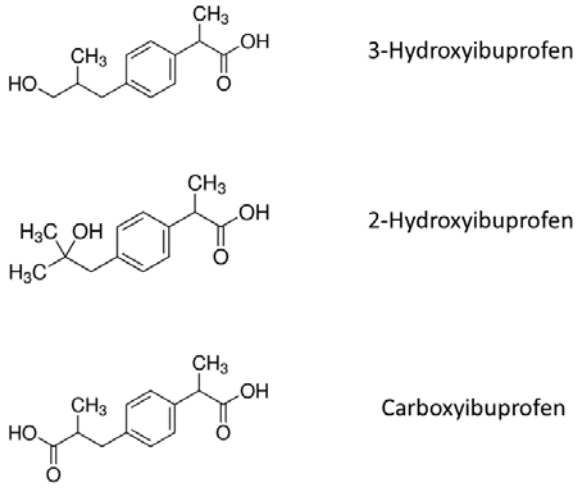

Figure 2.7 –Transformation products of IBP.

2.4.2. Occurrence of TPs in environment

In this part, the occurrences of CAF TPs and profen TPs in previous studies were summarized.

2.4.2.1. Occurrence of CAF TPs

CAF was frequently detected in the influents of WWTPs as presented above. SAT and AS treatment have the potential on the removals of CAF. According to the previous works, CAF degrading bacteria belonged to *Pseudomonas* [171, 172], and theobromine were formed by *Pseudomonas* strain [173]. CAF degradation was also observed in fungi such as *Stemphyllium* sp. [174], *Penicillium* sp. [175] and *Aspergillus* sp. [176], and the initial degradation product in fungi has been found to be theophylline [157].

For the occurrences of CAF TPs, Ghoshdastidar et al. [177] reported that the concentration of paraxanthine (18.2 μg/L) was high at WWTPs effluents in Canada. However, to my best knowledge, no literature reported the transformation of CAF in SAT.

2.4.2.2. Occurrence of profen TPs

Profens can be transformed by metabolism in human. For example, only 15% of IBP is excreted as IBP, while 26% is excreted as hydroxyl IBP and 43% as carboxyl IBP [135]. Though no significant transformation along the sewer system and primary clarification was observed, Weigel et al. [178, 179] reported that the concentrations of IBP metabolites (hydroxyl IBP and carboxyl IBP) in an aquifer were three times higher than the concentration of IBP in surface waters (rivers, lakes). Hydroxylated and carboxylated metabolites were considered as important TPs in biological treatments.

For the transformation process, biodegradation tests of KTP and NPX in the lab-scale activated sludge treatment were performed by other researchers [81, 180, 181], and they showed that aldehyde reduction of KTP and ether cleavage of NPX were the dominate transformation processes in their initial transformations.

For the behavior of TPs in WWTPs, Bendz et al. [51] reported that high removal efficiencies was not only expected for carboxyl IBP (96%), but also for hydroxyl IBP (95%). Buser et al. [182] reported that both metabolites of IBP are easy to be eliminated during sewage treatment. In most environmental samples, hydroxyl IBP was the dominant TP of IBP. However, to my best knowledge, few researches reported the biodegradation types of profens, and no research reported the occurrences of profen TPs in SAT.

2.5. Summary

AS treatment has limitation on the complete removals of micropollutants. SAT is an alternative treatment to wastewater reclamation after AS treatment, but the removal mechanisms of micropollutants in SAT are not clear. Thus, the feasibility of cooperation of AS treatment and SAT should be evaluated by the comparative study on the removal mechanisms in AS treatment and SAT.

Among micropollutants, PPCPs are an ideal group of compounds for evaluating the removal mechanisms of micropollutants because of their varieties of chemical properties. In addition, PPCPs could cause potential risk on human health and ecosystem.

Operating condition such as packing materials, HRT and vadose condition affect the removal performance of SAT and the treatment capacity of SAT. Thus, the balance of operating condition and the removals of micropollutants in SAT should be evaluated for practical use.

CAF possesses three methyl functional groups and profens possess carboxyl groups in common, so they were chosen as transformation indicators in the research of TPs. Demethylation of CAF and transformation types of profens can support the transformation of related micropollutants in AS treatment and SAT.

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Chapter 3 Comparative study on the removal mechanisms of PPCPs between SAT and AS system

3.1. Introduction

As presented in Chapter 2, both AS treatment and SAT have the potential to remove micropollutants. However, their removal characteristics were different because of the difference of treatment media (activated sludge and soil, respectively), operating conditions and water quality of feeding water. Sei et al. [1] mentioned that soil degraded 1, 4-dioxane more effectively than activated sludge because different types of 1,4-dioxane degrading bacteria were expected in soil. However, to the best of my knowledge, no researchers made a comparison between AS treatment and SAT on the removals of micropollutants with respect to their properties. In this study, SAT is applied after AS treatment for wastewater reclamation, so the role of SAT after AS treatment need to be evaluated based on the differences of removal mechanisms between SAT and AS treatment. PPCPs were selected as the indicators of micropollutants to evaluate the removal mechanisms in SAT because of their wide variety of properties.

Biodegradation and sorption contribute to the removal of pollutants in SAT [2]. Sorption occurred because of the interaction between micropollutants and silicate clays, metal oxides, and soil organic matter (SOM) in soil [3]. Furthermore, biomasses including activated sludge can affect the sorption of micropollutants [4]. Stringfellow et al. [5] mentioned that bacterial biomass contributed to the sorption and biodegradation on micropollutants. Thus, the effect of biomass in wastewater effluent on the sorption in SAT should be evaluated. In addition, biodegradation is expected in SAT as well as in AS treatment, but these two systems have different living environments for microorganisms, indicating possible difference of biodegradation in these two systems. Usually, SAT is applied after AS treatment, so the biodegradation in SAT and AS treatment should be evaluated to understand the possibility of the cooperation of AS treatment and SAT.

In this chapter, firstly, the role of sorption in SAT was evaluated by evaluating the difference of removals of fluoroquinolones (LVFX, ERFX, CPFX, and NRFX) between non-sterilized weathered granite soil (hereinafter called as WGS) columns and sterilized WGS columns. Secondly, the influence of biomass on sorption coefficients (K_d) was

evaluated by comparing the sorption isotherm of fluoroquinolones between WGS in SAT columns and autoclaved WGS. Thirdly, the range of biodegradation between SAT and AS treatment was compared by the difference of removal efficiencies of PPCPs between SBR and WGS columns. Finally, the removal kinetics of biodegradable PPCPs between these two systems indicated the effects of chemical structures on biodegradation. Through the comparison of SAT columns and SBR, the difference of biodegradation between SAT and AS treatment was evaluated.

3.2. Materials and methods

In this chapter, firstly, the contribution of biodegradation and sorption on the removals of PPCPs were evaluated by SAT column experiments. Secondly, the characteristics of biodegradation (i.e., biodegradation rate) were evaluated by soil-batch treatment experiment with the addition of PPCPs. Thirdly comparative experiment with SBR and SAT columns were performed. In the SAT column experiments, NaN_3 was used for sterilizing columns, and removal efficiencies in sterilized and non-sterilized columns were compared. In the soil batch-treatment experiment, firstly, the sorption amounts of fluoroquinolones (LVFX, ERFX, CPFX, and NRFX) in autoclaved WGS and WGS in SAT were compared to understand the influence of biomass on the sorption in SAT. Secondly, the kinetic experiment of biodegradable PPCPs (FNP, NPX, KTP, IBP, CFB, GFB, BZF, and FSM) in SAT was performed as well as the kinetics experiment of these PPCPs in SBR. In the comparative experiment, the differences of PPCP removals in SBR and SAT column were evaluated to understand the difference of biodegradation between these two systems. The relationship among different experiments in this chapter is shown in Figure 3.1.

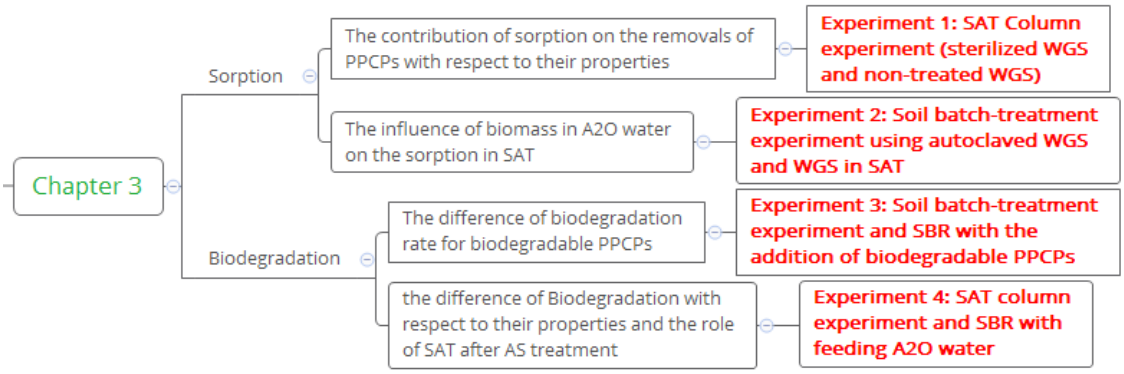

Figure 3.1 –Relationship among the different experiments in this chapter.

3.2.1. Reagents

In this study, 42 compounds were selected as target PPCPs as mentioned in Chapter 2. CAF and CTC were purchased from Cosmo Bio Co., Ltd.. IPP and LVFX were purchased from Tokyo Chemical Industry Co., Ltd.. ATP, LCM, ATL, AZM, CPFX, DTZ, ERFX, FNP, FSM, GFB, MFA, OTC, SDIME, TC, IBP, SMETH, and TRM were

purchased from Funakoshi Co., Ltd.. All other compounds were purchased from Wako Pure Chemical Co., Ltd. For stock solution, CAM and SMT were dissolved into acetone [6]. CPFX, LVFX, NRFX, and ERFX were dissolved into the mixture of water and acetonitrile (5:5), and all the other compounds were dissolved into acetonitrile. For the analysis of PPCPs with a LC-MS/MS system, formic acid, methanol, and acetonitrile (LC-MS grade, Wako Pure Chemical Co., Ltd) were used for the preparation of mobile phases. All the aqueous solutions were prepared with ultra-pure water produced with Millipore Academic-A10 system (hereinafter called as MQW).

For the preparation of nutrient solution for SBR, glucose, NaN_3, NaCl, $CaCl_2$, KH_2PO_4, NH_4Cl, $MgSO_4$, $Fe_2(SO_4)_3$, $MnSO_4$, $CuSO_4$, and $CoCl_2$ were purchased from Wako Pure Chemical Co., Ltd..

3.2.2. Column experiments

WGS collected in Shiga Prefecture was used as the packing material in SAT. Shiga Prefecture is located in the west of Japan, and WGS is a typical soil in this area. In addition, Toyoura standard sand (TS) was used for the comparison of WGS, and it is a typical sand in Japan [7]. The characteristics of selected packing materials (WGS, sand and TS) were presented in Table 3.1 The cation exchange capacity (CEC) and total organic carbon (TOC) of WGS were higher than those of sand and TS.

Table 3.1 –Characteristics of WGS, sand and Toyoura standard sand.

	WGS	Sand	Toyoura standard sand (TS)
Moisture content (%)	1.1	0.567	0.0654
pH	7.41	6.64	6.87
CEC(meq/100mg)	15.6	2.39	0.192
TOC	0.0159	0.0094	0.0144
Porosity	0.40	0.44	0.43
Particle size distribution (%, Texture: sand)	>99	>99	100
Specific surface (m^2/g)	5.95	2.40	0.812

For the parallel comparison of the removal efficiencies of PPCPs between sterilized WGS columns and WGS columns, four lab-scale acrylic columns (i.d., 8 cm; H, 100 cm) were used to simulate SAT. The columns consisted of the bottom layer (gravel level, 10 cm), the soil layer (WGS, 70 cm), and vadose zone (10 cm). The feed water for these columns was pumped with peristaltic pumps (SJ1220, ATTA). Because Mansell et al.[8] mentioned that 5 days was sufficient for the biodegradation of biodegradable organic carbon in SAT, and the hydraulic retention time (HRT) of both lab-scale columns were set at 7 days. The operation of non-sterilized and sterilized WGS columns started in April 2013. A schematic diagram of SAT columns and SBR is shown in Figure 3.2.

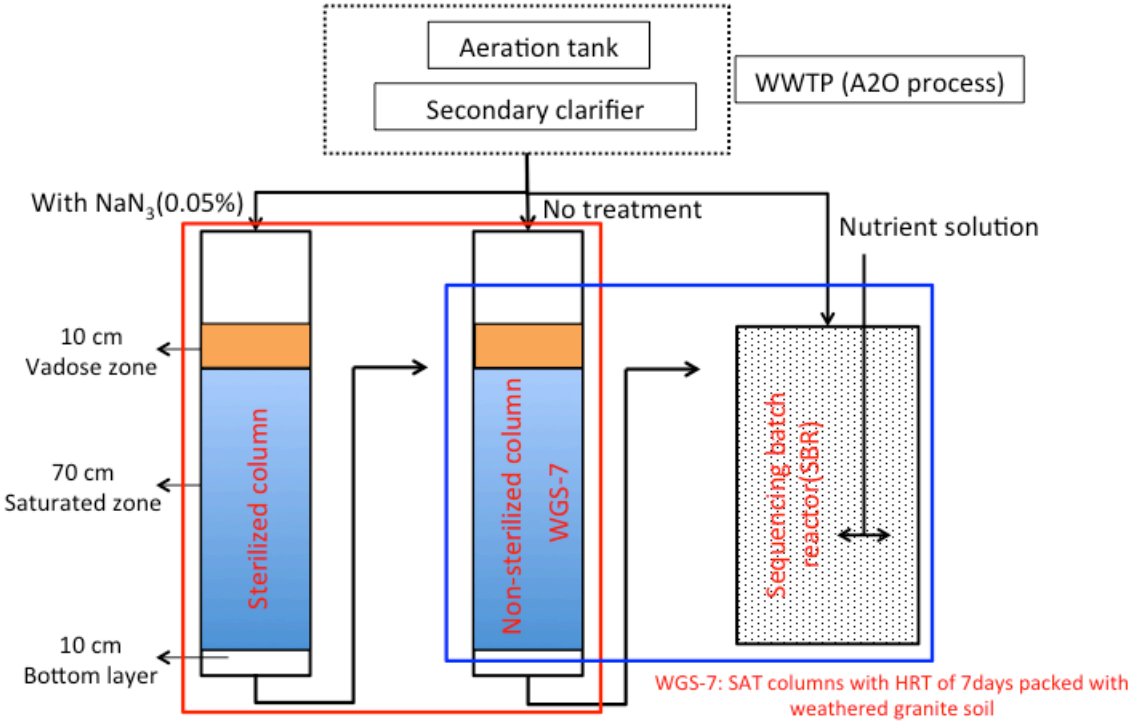

Figure 3.2 –Schematic diagrams of SAT columns and SBR.

3.2.3. SBR

A lab-scale SBR (i.d., 15 cm; H, 50 cm, water volume 12 L) was operated to simulate AS treatment. The seed sludge for this SBR was collected from an aeration tank in a WWTP in Kyoto. The influents and the effluents of SBR were pumped with peristaltic pumps (FP-300-1515, AS ONE corporation). Sludge and feed water were mixed with a mixing propeller of an overhead stirrer (RW16, IKA Corporation). The SBR processes consisted of the following steps: (Feeding step) 3 L of influent was pumped into the reactor at 300 mL/min for 10 min; (Aeration step) an overhead stirrer and an air pump were turned on, then the mixture was aerated for 6 h; (Settling step) after aeration, mixture and aeration were stopped for 1.5 h to settle the sludge; (Discharging step) after settlement, 3 L of supernatant was discharged from the reactor at 150 mL/min for 20 min.

After an intermission of 10 min, the sample cycle was repeated. The total operation time of one cycle was 8 h, and this cycle was repeated 3 times a day.

In order to maintain the operation of SBR (i.e., to support the metabolism of biomass in activated sludge), nutrient solutions were added into the feed water referring to the previous studies [9, 10] and the water qualities of influent in target WWTP (Table 3.2). The nutrient solution consisted of glucose (180 mg/L), NaCl (58.5 mg/L), $CaCl_2$ (22 mg/L), KH_2PO_4 (20.4 mg/L), NH_4Cl (78 mg/L), $MgSO_4$ (94 mg/L), trace elements ($Fe_2(SO_4)_3$, $MnSO_4$, $CuSO_4$, and $CoCl_2$, 0.2 mg/L each). The operating conditions in SBR and A_2O process in the WWTP are also shown in Table 3.2.

Table 3.2 –Operating conditions in SBR and A_2O process in the WWTP.

Parameters	SBR	A_2O process in the WWTP
pH	7.0-7.6	7.1
Temperature	20.0 °C	17.0 °C
HRT	6 hrs (O)	2.4/2.4/7.3 hrs (A/A/O)
SRT	40 days	16 days
DOC of influent	72.4-80.3 mg/L	-
DOC of effluent	5.9-10.3 mg/L	-
COD of influent	-	63 mg/L
COD of effluent	-	6.5 mg/L
BOD of influent	-	140 mg/L
BOD of effluent	-	2.3 mg/L
SVI	2.5-3	-
MLSS	1875	1510
MLVSS	1057	1105
MLVSS/MLSS	0.56	0.73

In kinetics experiments, each sample was collected at each sampling time followed by filtration. In the comparative experiment, the influents and effluents of SBR were sampled to evaluate the removal efficiencies of PPCPs in SBR.

3.2.4. Soil batch-treatment (SBT) experiment

The soil for SBT experiments was collected from the surface of the WGS pilot-scale reactor in the target WWTP. The soil samples were stored at 4 ^oC until analysis or use (no more than 2 weeks). The soil batch-treatment experiments were conducted with a series of flasks, in which 30 g of the soil was placed. FNP, NPX, KTP, IBP, CFB, GFB, BZF, and FSM were chosen as target compounds for biodegradation experiment because of their high biodegradabilities in SAT. In this experiment, A_2O water fortified with target PPCPs (1 μg/L) was fed into SBT experiments. At each sampling time, the suspension in each flask was filtrated with glass filter, and the concentrations of PPCPs in the filtrate were determined by LC-MS/MS.

3.2.5. Sorption experiment

Fluoroquinolones were selected as the target compounds to evaluate the influence of biomass on sorption in SAT because of their strong sorption and slow biodegradation in SAT. Sorption experiments of fluoroquinolones (i.e., LVFX, ERFX,CPFX, and NRFX) spiked to WGS in SAT and autoclaved WGS were conducted in accordance with the OECD test guideline [11].

For evaluating the influence of the biomass on sorption, control experiments should be operated without biomass. In this study, autoclaved WGS was chosen as control samples. Lotrario et al. [12] reported that autoclaving slightly increased the pH and cation exchange capacity of the soil, but had little effect on the soil organic matter. In

addition, Salonius et al. [13] demonstrated that autoclaving are not likely to have a significant effect on the adsorption characteristics of soil. Thus, autoclaved WGS can be used as the control sample for evaluating the effect of biomass on the sorption.

The concentrations of soil to give an approximately 50% adsorption for fluoroquinolone in 24 h were 400 and 600 mg/L WGS in SAT and autoclaved WGS (Figure 3.3). After determining the concentrations of soil, sorption isotherm experiments were operated. The initial concentrations of the each fluoroquinolone in the aqueous phase were set to 20, 40, 60, 80, and 100 μg/L, and contact times with soil were 0, 1, 3, 8, 16, and 24 h. The pH was adjusted to 7.0 with phosphate buffer (0.02 M), and the concentration of each fluoroquinolone monitored for 24 h.

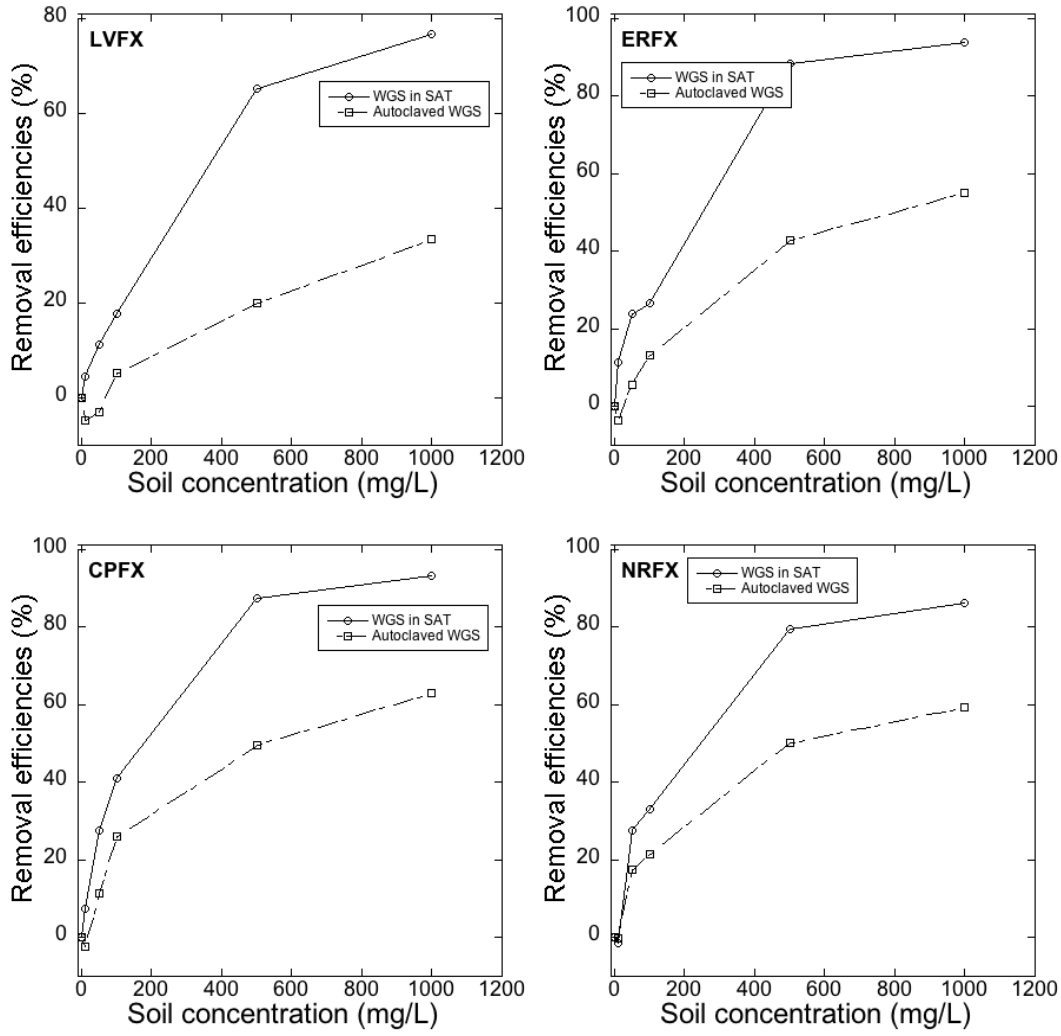

Figure 3.3 –Removal efficiencies of LVFX, ERFX, CPFX, and NRFX with different the concentration of soils in the sorption experiment.

3.2.6. Feed water

Feed water was sampled at Toba WWTP (a WWTP in Kyoto City, and its treatment capacity was 914000 m^3/day). The treatment process of Toba plant was anaerobic-anoxic-oxic (A_2O) process. The PPCP concentrations of the effluent from A_2O tank were detected from June 2013 to December 2013 (June 2nd, June 6th, June 17th, July 16th, July 28th, August 1st, August 12th, August 26th, September 8th, November 29th, and December 4th) in order to calculate their average concentrations in A_2O water (n=11). After collection, samples were filtrated by 100 μm filters and stored at 4 °C. Effluents from SAT columns were sampled from July 2013 to November 2013 (July 14th, July 17th, July 28th, August 1st, August 6th, August 12th, August 19th, August 26th, September 8th, November 6th, and November 29th) (n=11). Effluents from SBR were sampled from July 2013 to November 2013 (June 2nd, June 6th, June 17th, July 14th, July 28th, August 6th, August 12th, August 19th, August 26th, September 8th, November 6th, and November 29th) (n=12). Referring to the works of Takabe et al.[14], the water quality of A_2O water is shown in Table 3.3.

Table 3.3 –Water qualities of A_2O water.

pH	**DO(mg O_2/L)**	**DOC(mg C/L)**	**UV_{254}(cm^{-1})**
6.43 (6.37-6.50)	1.47(1.13-1.75)	3.68(3.31-4.19)	0.082(0.070-0.128)
DTP(mg P/L)	**DTN(mg N/L)**	**NH_4^+(mg N/L)**	**NO_2^-(mg N/L)**
0.400 (0.280-0.805)	4.43(3.87-4.89)	<0.02(<0.02-0.56)	0.046(0.014-0.080)
NO_3^-(mg N/L)	**Organic-N(mg N/L)**		
3.41(3.26-3.46)	0.92(0.53-0.97)		

3.2.7. Analytical methods

3.2.7.1. Pretreatment for PPCPs measurement

All the water samples were concentrated with solid phase extraction (SPE) after the filtration through glass fiber filters (ADVANTEC GF-75, 90 mm). The SPE cartridge (Oasis HLB cartridges (Waters), 225 mg bed) was conditioned with 5 mL of methanol and 5 mL of MQW at pH 3 (adjusted with hydrochloric acid). Samples were passed through the cartridges at a flow rate of 10 mL/min for 20 min. After drying the SPE cartridge with nitrogen gas, the retained compounds were eluted with 5 mL of methanol. The eluted solutions were evaporated to dryness under a gentle stream of nitrogen gas[6, 7]. The residues of A_2O water samples and SAT effluent samples were re-dissolved in 2 mL and 1 mL of 0.1% formic acid-acetonitrile (90/10, v/v), respectively.

The samples after concentration were analyzed by LC-MS/MS. In this study, a prominence 20A HPLC system (Shimadzu) was interfaced with 4000 QTRAP tandem

mass spectrometer (AB SCIEX). A C-18 column (Kinetex, 2.1×50 mm, 2.6 μm) was used for the negative mode analysis, and a Shim-pack VP-ODS column (Shimadzu, 4.6×150 mm, 4.6 μm) was used for the positive mode analysis. The recoveries of the target compounds were determined by fortifying standard compounds into A_2O water or SAT effluent. The fortified sample and the blank (i.e., A_2O water without fortification) were concentrated as described above. These samples were prepared in duplicate, and the recovery rate of each target compound was calculated with the following formula.

$$\text{Recovery rate} = \frac{\text{Conc. in the fortified sample} - \text{Conc. in blank}}{\text{Fortified Conc.}}$$

3.2.7.2. LC-MS/MS conditions for PPCPs measurement

In the LC-MS/MS measurement, multiple-reaction monitoring (MRM) mode was chosen for the quantification of target compounds. Operating conditions of LC-MS/MS were optimized by using standard solution in MQW. LC-MS/MS conditions, MRM parameters, and LC gradient for the separation of target compounds are shown in Tables 3.4-3.9.

Table 3.4 –LC-MS/MS conditions (positive mode).

LC condition	LC system	Prominence 20A (Shimadzu)
	Column	VP-ODS 4.6 μm 150×4.6mm(Shimadzu)
	Column temperature	40 °C
	Sample cool temperature	10 °C
	Inject volume	20 μL
	Mobile phase	0.1% formic acid/acetonitrile
	Flow rate	0.8 mL/min
	Measurement time	35 mins
MS/MS condition	MS/MS	4000 QTRAP(AB SCIEX)
	Ionization mode	ESI+
	Curtain gas	10 psi
	Ionspray voltage	5000 V
	Turbo ionspray temperature	600 °C
	Nebulizer gas pressure	60 psi
	Turbo gas pressure	50 psi
	Collision gas	6

Table 3.5 –LC-MS/MS conditions (negative mode).

	LC system	Prominence 20A (Shimadzu)
	Column	C-18 2.6 μm 50×2.1 mm (Kinetex)
	Column temperature	40 °C

LC condition	Sample cool temperature	10 °C
	Inject volume	10 μL
	Mobile phase	MQW/methanol
	Flow rate	0.4 mL/min
	Measurement time	22 min
MS/MS condition	MS/MS	4000 QTRAP(AB SCIEX)
	Ionization mode	ESI-
	Curtain gas	25 psi
	Ionspray voltage	-4000 V
	Turbo ionspray temperature	550 °C
	Nebulizer gas pressure	60 psi
	Turbo gas pressure	40 psi
	Collision gas	6

Table 3.6 –MRM conditions (ESI+).

	Q1/Q3	DP (V)	EP (V)	CE (V)	CXP (V)
LVFX	362.053/318.100	76	10	29	18
ERFX	360.112/316.200	91	10	27	8
NRFX	320.107/276.300	86	10	27	24
CPFX	332.069/288.200	66	10	27	22
SDIME	310.960/156.000	71	10	33	10
ATL	267.129/145.000	81	10	37	10
TC	445.096/409.900	61	10	27	10
DTZ	415.080/178.200	61	10	31	12
LCM	407.081/126.200	76	10	43	10
CBM	237.185/194.200	66	10	29	0
CAM	748.468/158.100	96	10	41	22
CRT	204.147/69.100	61	10	39	4
DEET	192.190/119.200	61	10	25	18
ERY	734.537/158.000	126	10	55	14
SLP	342.064/112.100	101	10	37	6
CAF	195.112/138.000	66	10	27	8
SMT	279.179/92.000	56	10	43	14
AZM	749.520/591.400	151	10	45	16
CTC	479.099/462.100	61	10	29	12
TRM	291.033/230.000	91	10	33	12
IPP	231.078/189.100	76	10	31	8
ATP	189.026/56.100	71	10	53	8
AMP	350.005/106.000	66	10	33	18

BP	183.019/104.900	56	10	23	18
OTC	461.046/426.100	46	10	27	12
PRM	219.150/162.100	61	10	19	14
DSP	340.177/239.000	41	10	25	18
TEP	181.205/124.000	21	10	27	8

Table 3.7 –MRM conditions (ESI-).

	Q1/Q3	DP (V)	EP (V)	CE (V)	CXP (V)
IBP	205.078/161.100	-40	-10	-10	-23
ACEAM	149.946/106.900	-65	-10	-24	-7
GFB	249.025/120.800	-45	-10	-30	-9
FSM	328.878/285.000	-50	-10	-20	-15
SMETH	251.912/155.800	-45	-10	-20	-9
ASP	178.923/136.800	-25	-10	-10	-1
FNP	240.862/196.900	-40	-10	-10	-9
MFA	239.853/195.900	-45	-10	-22	-11
BZF	360.000/153.900	-45	-10	-46	-25
CFB	212.567/127.100	-35	-10	-24	-7
DCF	293.898/250.100	-15	-10	-18	-1
TRC	287.144/35.300	-30	-10	-30	-7
KTP	252.888/209.000	-30	-10	-10	-15
NPX	228.857/168.900	-30	-10	-34	-13

Table 3.8 –LC gradient condition (ESI+).

Time	Mobile phase (%)	
	0.1% formic acid	Acetonitrile
0	90	10
1	90	10
10	82	18
11	75	25
17	65	35
25	40	60
27	2	98
33	90	10

36	90	10

Table 3.9 –LC gradient condition (ESI-).

Time	Mobile phase (%)	
	MQW	Methanol
0	90	10
1	90	10
4	60	40
15	30	70
16	5	95
21	5	95
22	90	10
25	90	10

3.2.7.3. Bacterial counts

In order to confirm the sterilization in the sterilized columns, general bacteria and heterotrophic bacteria were counted. Microorganisms were separated from the soil and sand, and the biomass suspensions were prepared as follows. Firstly, microorganism in the sand and soil were dispersed from sand and soil with a Waring blender (Ace homogenizer AM-3, Nihonseiki, Japan). Secondly, 10 g (wet weight) of sand or soil sample was mixed with 40 mL of phosphate buffer (50 mM, pH = 7.4), and the mixture was homogenized with the blender for 3 min. Thirdly, the homogenate was centrifuged at 800 rpm for 1 min (KUBOTA 5200, Kubota, Japan) to remove the particles, and the supernatant was used in the following measurement as biomass suspension.

23.5 g of standard methods agar (NIHON PHARMACEUTICAL CO., LTD.) was dissolved into 1 L of MQW for the general bacteria. After dissolution, the solution was autoclaved at 121 ^{o}C for 20 min. R2A agar was used for the heterotrophic bacteria counting, and 18.2 g of R2A agar was dissolved into 1 L of MQW, followed by autoclaving at 121 ^{o}C for 20 min. Biomass suspension (1 mL) and its diluted samples were mixed with agar solution for plate culture of bacteria. The plates for general bacteria were incubated at 37 ^{o}C for 24 hrs, and the plates for heterotrophic bacteria were incubated at 20 ^{o}C for 7 days before counting colonies.

3.2.8. Preliminary experiments

3.2.8.1. Lower limit of quantification

The concentrations with a signal-to-noise (S/N) ratio of 10 was considered as the lower limit of quantification [15]. If the concentration lower than 0.01 μg/L but the value of S/N was above 10, the lower limit of quantification (LOQ) was set to 0.01 μg/L.

A series of standard solutions were prepared by diluting the stock standard solution with MQW (0.05, 0.1, 0.5, 1, 5, 10, 50, 100, and 200 μg/L). The relationship between peak areas and the designated concentrations of each compound was used to calculate the PPCP concentration.

In this study, because of the lack of internal standard compounds for each PPCP, and limited volume of SAT effluents, the standard addition method was impractical. Kim [16] mentioned that MQW spiked target PPCPs were used directly to LC-MS/MS for PPCP quantification. In this study, the absolute calibration was chosen to determine the concentrations of PPCPs, and the matrix effect was separately evaluated in **3.2.8.2**.

Typical chromatograms in positive and negative modes were shown in **Figures 3.4 and 3.5**, respectively, and target compounds were well separated in LC columns. Five points above the lower LOQ were used for calibration. To account for low concentration points (0.05, 0.1, 0.5 and 1 μg/L), 1/*x* was selected as the weighting function for regression analysis.

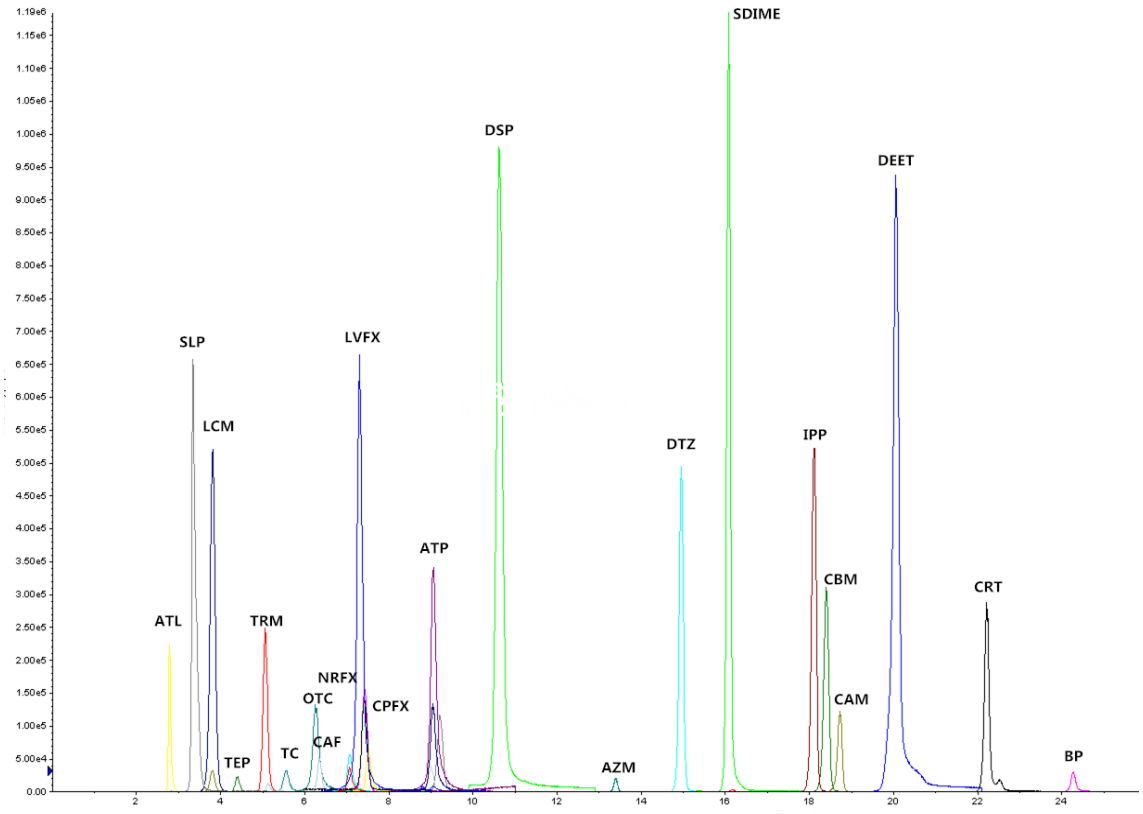

Figure 3.4 –The chromatogram of the target PPCPs in positive mode (10 µg/L).

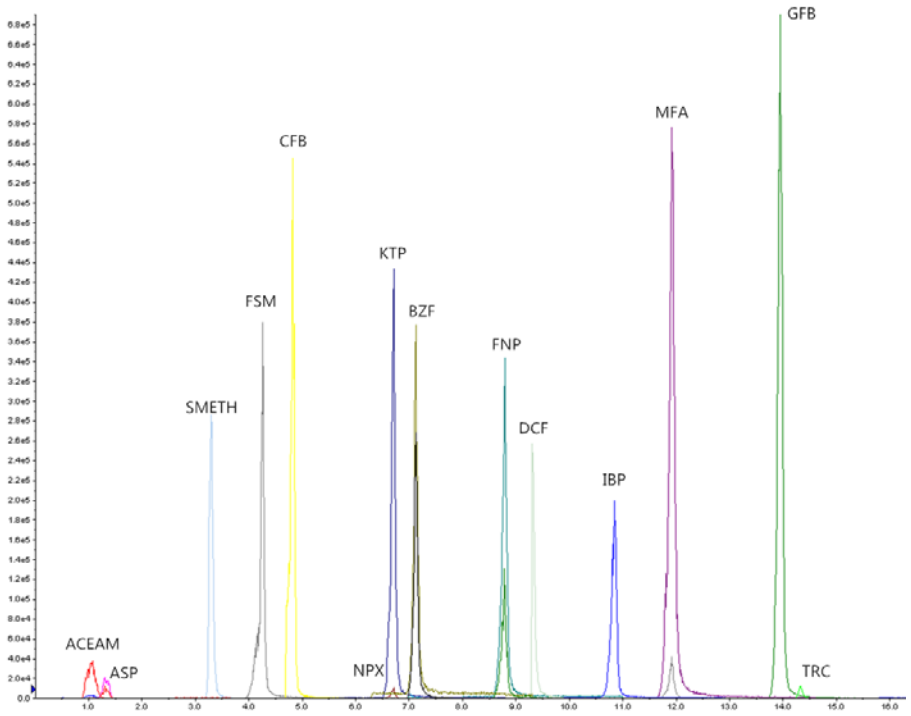

Figure 3.5 –The chromatogram of the target PPCPs in negative mode (10 µg/L).

3.2.8.2. Matrix effects

In order to evaluate the matrix effect of the A_2O and SAT samples, the peak areas in MQW and A_2O water were compared in the following way: Stock standard solution was added into MQW to make the concentrations 10, 50, and 100 µg/L, respectively. Because concentrations of PPCPs in A_2O water were several hundred ng/L, standard solutions (10, 50, and 100 µg/L, respectively) to evaluate the matrix effect was prepared by A_2O water. Samples with known concentrations were measured by LC-MS/MS, and their peak areas were compared (Figure 3.6).

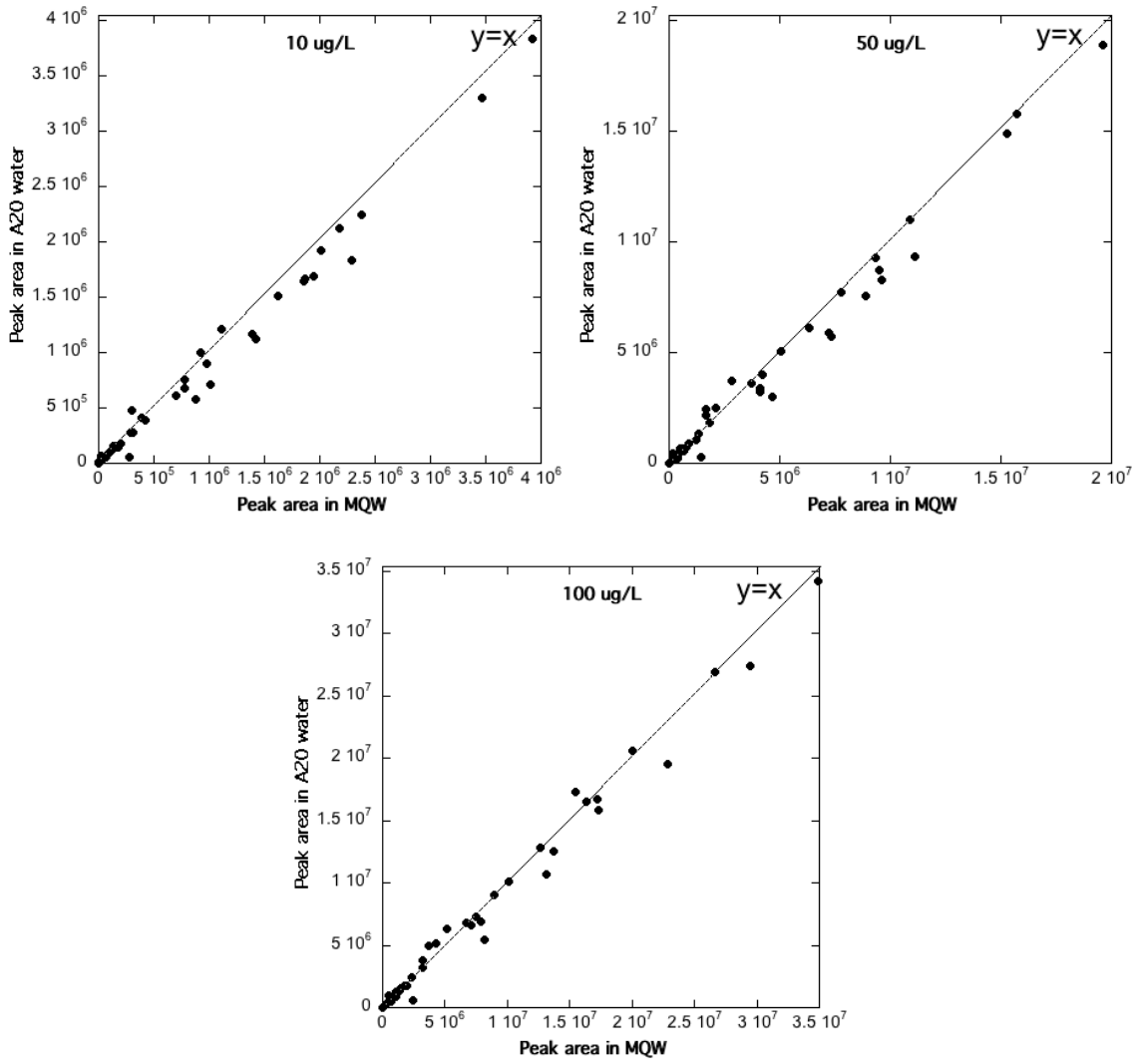

Figure 3.6 –The difference of peak areas between MQW and A_2O water with spiking standard compounds.

In Figure 3.6, the peak areas of PPCPs in A_2O water were not different from the peak areas of MQW. This result indicates that matrix effect is negligible for A_2O water, and the absolute calibration method was available in this study. Furthermore, in order to reduce the influence of injection volume or possible degradation of the standard solution, the standard solution for measurement was prepared before measurement, and the sensitivity of LC-MS/MS was checked before measurement.

3.2.8.3. Recoveries of PPCPs for A_2O water and SAT effluent

With the optimized conditions mentioned above, the recoveries of PPCPs in water samples were determined by spiking different concentrations of PPCPs (10 ng/L and 100 ng/L, n=5) in 300 mL samples of A_2O water and SAT water. The differences of peak

areas between spiked samples and blank samples were used for the calculation of recoveries.

The average recoveries of target PPCPs were shown in Figure 3.7 and the LOQ were shown in Table 3.10. Average recoveries of PPCPs in A_2O water and SAT effluents were 61.06% (ranged from 25.67% (CPFX) to 121.67% (BP)) and 60.62% (ranged from 22. 05% (SDIME) to 146.00% (BP)), respectively.

In addition, LOQ of target PPCPs without sample concentration ranged from 0.06 μg/L (GFB) to 1.64 μg/L (CTC). Compared with the works of Kim [15] and Ashton et al. [15, 17], the recoveries and LOQ in this study were acceptable for PPCPs measurement.

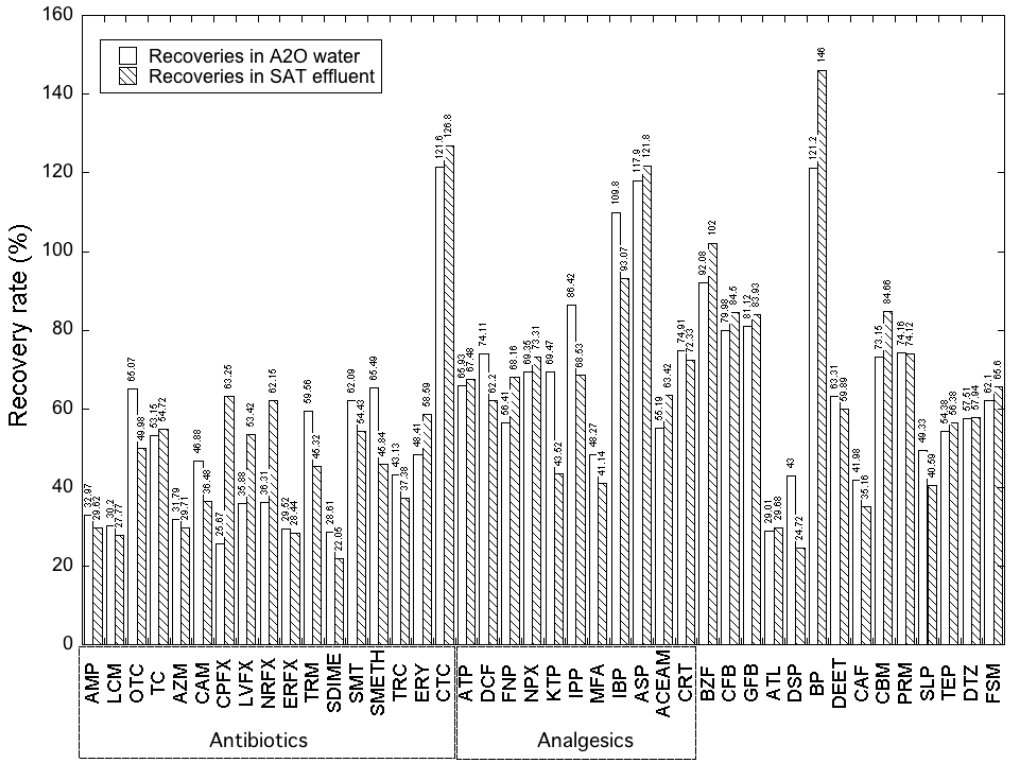

Figure 3.7 –The recoveries of PPCPs in A_2O water and SAT effluent.

Table 3.10 –LOQ of target PPCPs without sample concentration.

Compounds	LOQ (μg/L)	Compounds	LOQ (μg/L)	Compounds	LOQ (μg/L)
AMP	0.30	LCM	0.17	OTC	0.15
TC	0.09	AZM	0.31	CAM	0.11
CPFX	0.39	LVFX	0.28	NRFX	0.34

ERFX	0.34	TRM	0.08	SDIME	0.17
SMT	0.08	SMETH	0.08	TRC	1.16
ERY	1.03	CTC	1.64	ATP	0.08
DCF	0.07	FNP	0.89	NPX	0.72
KTP	1.29	IPP	0.06	MFA	0.21
IBP	0.91	ASP	0.85	ACEAM	0.91
CRT	0.13	ATL	0.34	DSP	0.12
BZF	0.11	CFB	0.13	GFB	0.06
DEET	0.16	BP	0.04	CAF	0.16
CBM	0.07	PRM	0.67	SLP	0.10
TEP	0.23	DTZ	0.09	FSM	0.16

3.2.8.4. The performance of sodium azide on sterilization

In order to separate the biodegradation and sorption in SAT, biodegradation needs to be suppressed in the sterilized column, and the removals of PPCPs in the sterilized columns were compared with those in the non-sterilized columns to understand the roles of biodegradation and sorption in SAT. Chefetz et al.[18] mentioned that NaN_3 is one of the biocides commonly used to inhibit the microbial growth in experiments. Thus, NaN_3 was used as a sterilant to maintain the sterilization in column. Two columns were fed with A_2O water with 0.05% sodium azide (NaN_3) for sterilization. In order to ensure the sterilization in the sterilized column, the general bacteria number and heterotrophic bacteria number in the sterilized and non-sterilized columns were detected to check the sterilization. The differences of bacterial population between the sterilized and non-sterilized columns (5.42×10^4 and 1.53×10^2 CFU/g for general bacteria numbers and 2.35×10^6 and 1.33×10^3 CFU/g in non-sterilized and sterilized columns, respectively) indicated that the sterilization of NaN_3 in columns was effective. Biodegradation was weak expected in the sterilized columns compared with that in the non-sterilized columns.

3.2.8.5. The influence of sodium azide on the measurement of PPCPs

The influence of NaN_3 on PPCPs measurement was evaluated in this part. In Figure 3.8, the concentrations of PPCPs in the SAT effluent and A_2O water with the addition of NaN_3 were compared with those without NaN_3. The results showed that NaN_3 did not influence the measurement of PPCPs in LC-MS/MS.

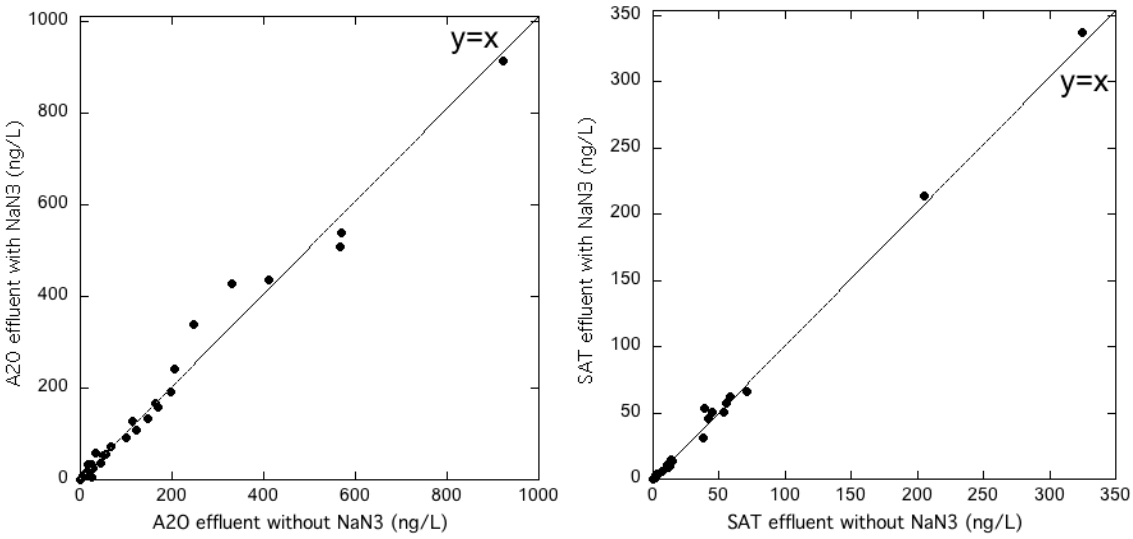

Figure 3.8 –The influence of NaN_3 on the measurement of PPCPs in A_2O water and SAT effluent.

3.2.8.6. Biomass measurement

Based on the works of Findlay et al. [19] and Rauch et al.[20], phospholipid concentration in media (i.e., soil and sludge) was measured as an indicator of biomass concentration with minor modification as follows. For soil samples, triplicates of 1 g soil as wet weight were placed in 50 mL centrifuge tubes with triple seal caps (Asahi Glass Co., LTD.). Phospholipid extraction from soil was performed in solution with phosphate buffer solution (PBS, 50 mM, pH = 7.4), methanol, and chloroform (PBS: methanol: chloroform = 6.4 mL: 16 mL: 8 mL). The tubes were tightly sealed and shaken for 24 h. Then 0.0306 M sulfuric acid and chloroform were added for a final ratio of water: methanol: chloroform = 0.9:1:1. The tubes were centrifuged at low speed (200 rpm), and phases were separated for 8 h. The upper phase was carefully removed without disturbing the chloroform layer. Eight milliliter of the lower chloroform layer was transformed into a glass vial. The chloroform extraction was completely dried down under nitrogen gas stream. Subsequently, 2 mL of saturated potassium persulfate agent (5% potassium persulfate in 0.36 N sulfuric acid) was added into each vial. The solution in the vials was mixed by a vortex mixer, and digested for 2 hrs at 120 ^{o}C. After cooling, the solution in vials was diluted into 100 mL, and the total phosphate in diluted solution was analyzed in automatic total phosphorus and total nitrogen meter (AACS II, BRAN+LUEBBE Co. Ltd).

Biomass in the soil of SAT column was 48.01 nmol/g, and biomass in SBR was 38.4 nmol/mL. In the kinetics experiment, the biomass in soil batch scale and SBR were 0.0048 mM and 0.0384 mM, respectively.

3.2.8.7. Stabilization of SAT columns

Before starting the column experiments, the steady state of SAT columns was confirmed by monitoring the concentration of DOC in the influent and effluents of the columns (Figure 3.9). In the initial phase (0-4 days), the average DOC concentration of non-sterilized columns was 5.82 mg/L, and the average DOC concentration of the sterilized columns was 8.43 mg/L. From 8 days, the DOC concentrations decreased in the effluent of SAT columns, and were at approximately 1 mg/L. Thus, after stabilization for 36 days, The SAT columns were assumed to enter a steady condition. In order to ensure the steady condition in SAT for the removals of micropollutants, samples of SAT effluents were collected after the stabilization of two months.

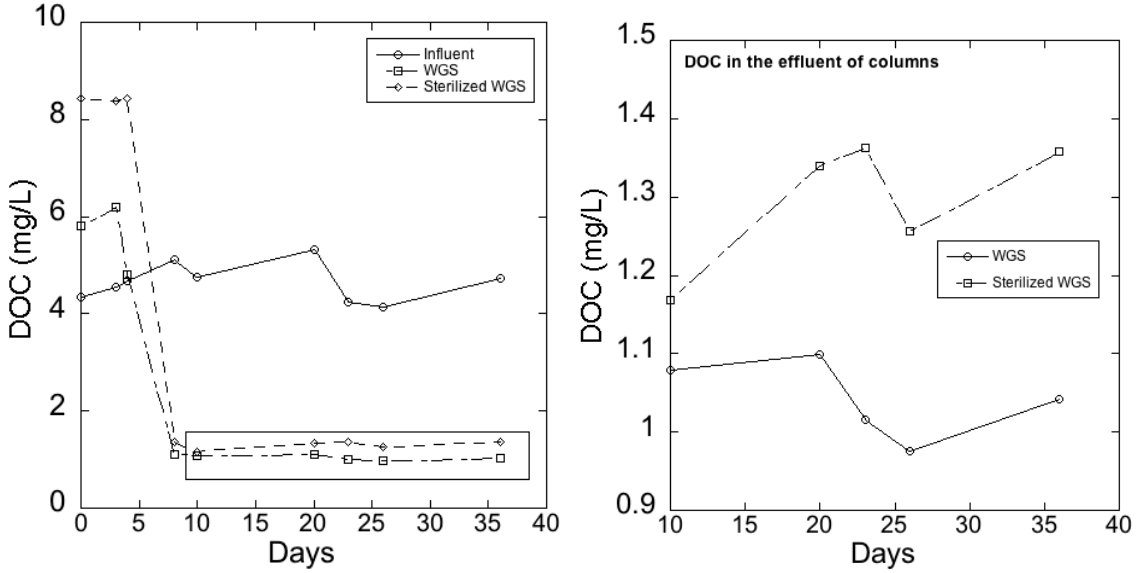

Figure 3.9 –Temporal changes in DOC for the influent and effluent of SAT sampled from the sterilized WGS and non-sterilized WGS columns.

3.3. Results

3.3.1. Occurrence of PPCPs in A_2O water

The total concentration of target PPCPs in the A_2O water was more than 5000 ng/L. The distribution of PPCPs in A_2O water is shown in Figure 3.10. The highest, lowest and average concentrations of the selected PPCPs in A_2O water were presented in Figure 3.11. Antibiotics and analgesics were the major PPCPs in A_2O water. For antibiotics, CAM, AZM, LVFX, and CPFX were observed at relatively high concentrations (769, 175, 28, and 23 ng/L, respectively), while ERFX was not detected in the A_2O water. For analgesics, CRT was the most abundant in the A_2O water (806 ng/L on average). In addition, KTP, DCF, MFA, ACEAM and NPX were also dominant (639, 80, 75, 17, and 34 ng/L respectively), while IBP was not detected in A_2O water. For other

groups of selected PPCPs, BZF, SLP, TEP, DEET, CAF, DSP and FSM were observed at high concentrations (193, 397, 108, 455, 94, 237, and 203 ng/L, respectively).

Because the concentrations of PPCPs fluctuated both for A_2O water [21] and SAT effluents during the monitoring period, the monthly average values (n=11) were used to calculate the removal efficiencies in the following parts.

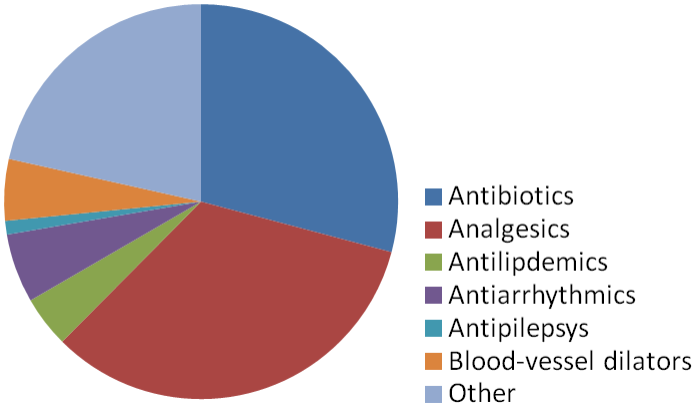

Figure 3.10 –The concentration distribution of PPCPs in A_2O water.

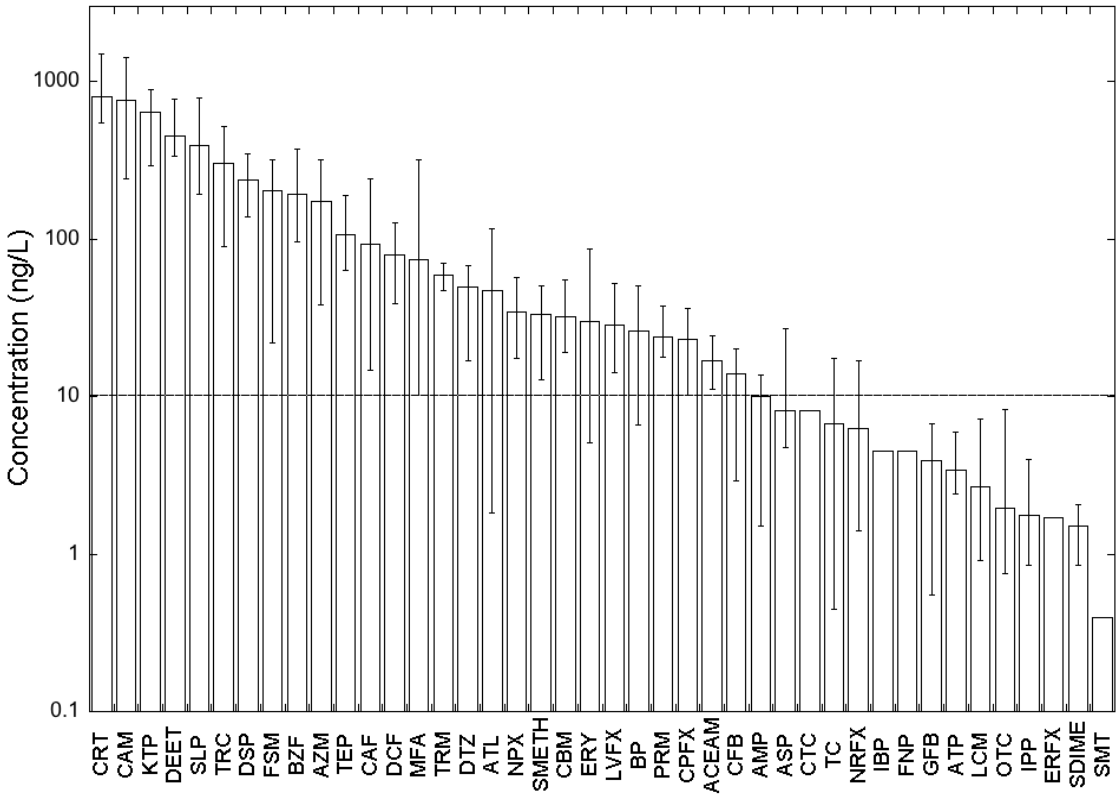

Figure 3.11 –The concentrations of PPCPs in A_2O water (n=11).

3.3.2. Sorption and degradation of target PPCPs in SAT columns

Based on the relatively high concentrations in A_2O water (>10 ng/L) and the detection frequencies, LVFX, CAM, LCM, AZM, SMETH, TRC, and TRM were selected as target antibiotics. ATP, IPP, DCF, KTP, NPX, MFA, CRT, and ACEAM were selected as target analgesics. BZF, CFB, and GFB were selected as target antilipidemics. PRM, CBM, SLP, FSM, DTZ, TEP, ATL, DSP, BP, DEET, and CAF were selected as other PPCPs.

The removal efficiencies of target PPCPs in the non-sterilized and sterilized columns were compared in Figure 3.12. The removals of target PPCPs were categorized as follows. (i) PPCPs removed effectively in both sterilized and non-sterilized columns; (ii) PPCPs removed effectively only in the non-sterilized columns; (iii) PPCPs cannot be removed sterilized and non-sterilized columns. In order to classify the removal efficiencies of PPCPs, the removal efficiencies were divided into four categories: highly efficient (>90%), efficient (80-90%), moderate (50-80%) and poor (<50%) [22-24].

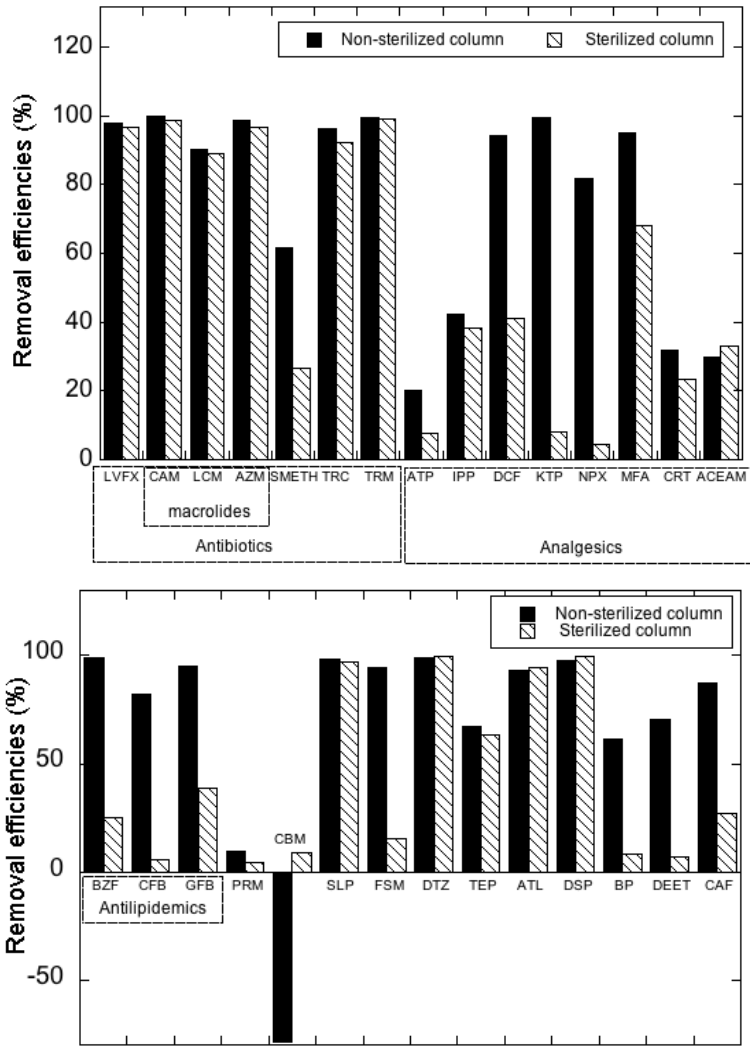

Figure 3.12 –PPCP removal efficiencies in non-sterilized and sterilized columns with an HRT of 7 days.

3.3.2.1. PPCPs removed effectively in both sterilized and non-sterilized columns

The removals of LVFX, CAM, LCM, TRC, AZM, SLP, DTZ, ATL, and DSP were highly efficient under both sterilized and non-sterilized conditions. Because the biodegradation in the sterilized columns was inhibited by NaN_3 [25], the removal mechanisms of these PPCPs in the sterilized columns was expected to be physicochemical interactions between soil and PPCPs. LVFX, a fluoroquinolone, was removed by 97.7% and 96.7% in the non-sterilized and sterilized columns, respectively. Similar removal trends were observed for CAM, LCM, and AZM (99.8%, 90.1%, and 98.8% in the non-sterilized column, 99.8%, 89.0%, and 96.5% in the sterilized columns, respectively). TRC, SLP, DTZ, ATL, and DSP were also efficiently removed in both the sterilized and non-sterilized columns.

3.3.2.2. Compounds removed effectively only in the non-sterilized column

DCF, KTP, NPX, MFA, BZF, CFB, GFB, FSM, and CAF were removed effectively in the non-sterilized columns, but their removal efficiencies were lower in the sterilized columns. KTP (K_{oc} =229-341 mL/g, pK_a =4.45), a NSAID, was removed by 8.2% and 99.6% in the sterilized and non-sterilized columns, respectively. NPX (K_{oc}= 330 mL/g, pK_a= 4.15), another NSAID, was also removed more efficiently in the non-sterilized columns (81.75%). MFA (K_{oc} = 880 mL/g, pK_a= 4.2) was removed highly efficiently in the non-sterilized columns and moderately effectively in the sterilized columns (68.2%). Among antilipidemics, the removal efficiencies of BZF (K_{oc}= 204 mL/g, pK_a= 3.83), CFB (K_{oc}= 204 mL/g, pK_a= 3.18), and GFB (K_{oc}= 430 mL/g, pK_a= 4.5) were 25.4%, 5.7%, and 38.6%, respectively in the sterilized columns, but the removal efficiencies of these compounds were high (98.8%, 82.1%, and 95.1%, respectively) in non-sterilized column. Among other PPCPs, the removals of FSM (K_{oc}= 302 mL/g, pK_a= 3.8, 7.5) and CAF (K_{oc}= 741 mL/g, pK_a= 10.4) were poor in the sterilized columns (15.1% and 27.3%, respectively), but those in the non-sterilized columns were 94.4%, and 87.2%, respectively.

3.3.2.3. Compounds persistent in both sterilized or non-sterilized column

The removals of ATP, IPP, CRT, PRM, and CBM were poor both in the sterilized and non-sterilized columns. These compounds contained amide functional groups in common, and were called as amide PPCPs [26]. Furthermore, the concentration of CBM in the non-sterilized columns increased.

3.3.3. Sorption isotherm of PPCPs in SAT soil

A_2O water contained rich biomass from activated sludge. Jacobsen et al. [27] mentioned that biomass in activated sludge adsorbed micropollutants such as 2,4-dichlorophenol (2,4-DCP). In this study, SAT was fed with A_2O water, and the biological

communities can grow in SAT columns with long-term operation with feeding A_2O water [2]. Compared with unused WGS, WGS in SAT was expected to contain rich biomass. Tigini et al. [28] reported that bio-sorption of polychlorinated biphenyls (PCBs) occurred in the soil with rich biological population. Thus, the influence of biomass in SAT on the sorption of micropollutants should be evaluated. According to the results above, fluoroquinolones (LVFX, ERFX, CPFX, and NRFX) were removed effectively in both the sterilized and non-sterilized columns, and sorption was likely to contribute to their removals in SAT. For evaluating the influence of the biomass on the sorption in SAT, soil batch-treatment experiments were conducted.

The concentrations of these fluoroquinolones after the contact with WGS in SAT and autoclaved WGS were compared in batch mode. WGS in SAT contained soil particles and biomass in SAT, and autoclaved WGS contained only soil particles. In Figure 3.13, LVFX, ERFX, CPFX, and NRFX were removed highly effectively in both WGS in SAT and autoclaved WGS. However, the concentrations of these compounds remained with autoclaved soil in this batch experiment were higher than those remained in WGS in SAT. The sorption isotherms between autoclaved WGS and WGS in SAT will be compared in the following part.

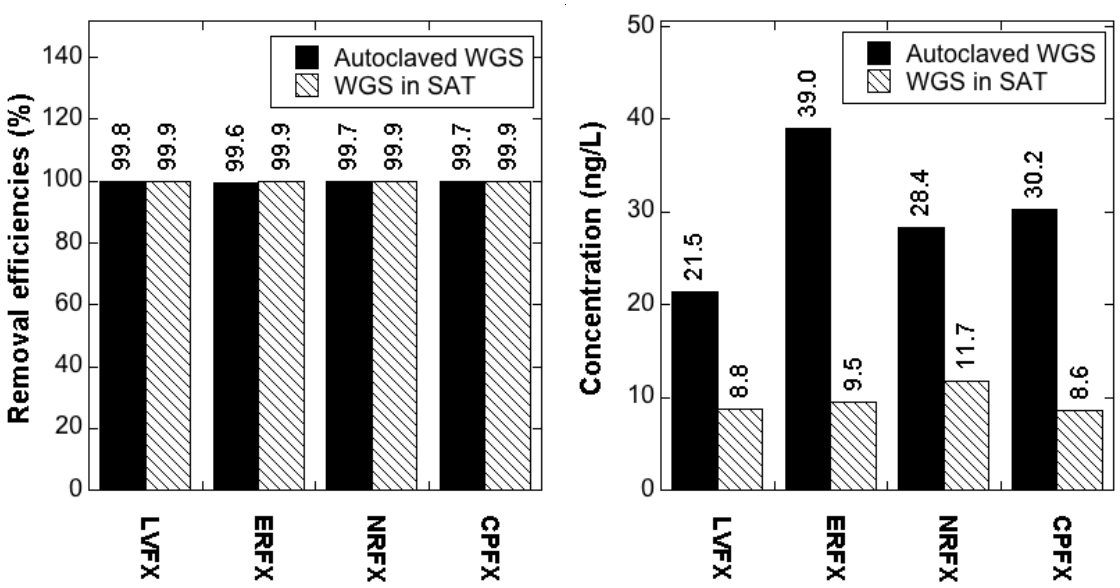

Figure 3.13 –The removal efficiencies (left) and concentrations (right) of fluoroquinolones in batch experiments with autoclaved WGS and WGS in SAT (10 µg/L).

Sorption isotherms were drawn based on the adsorbed concentration in the soil batch-treatment experiments (Figure 3.14). The partition coefficients (K_d) of LVFX, ERFX, CPFX and NRFX by WGS in SAT and autoclaved WGS are listed in Table 3.11. In summary, sorption coefficients of the fluoroquinolones in WGS in SAT were higher than those in autoclaved WGS.

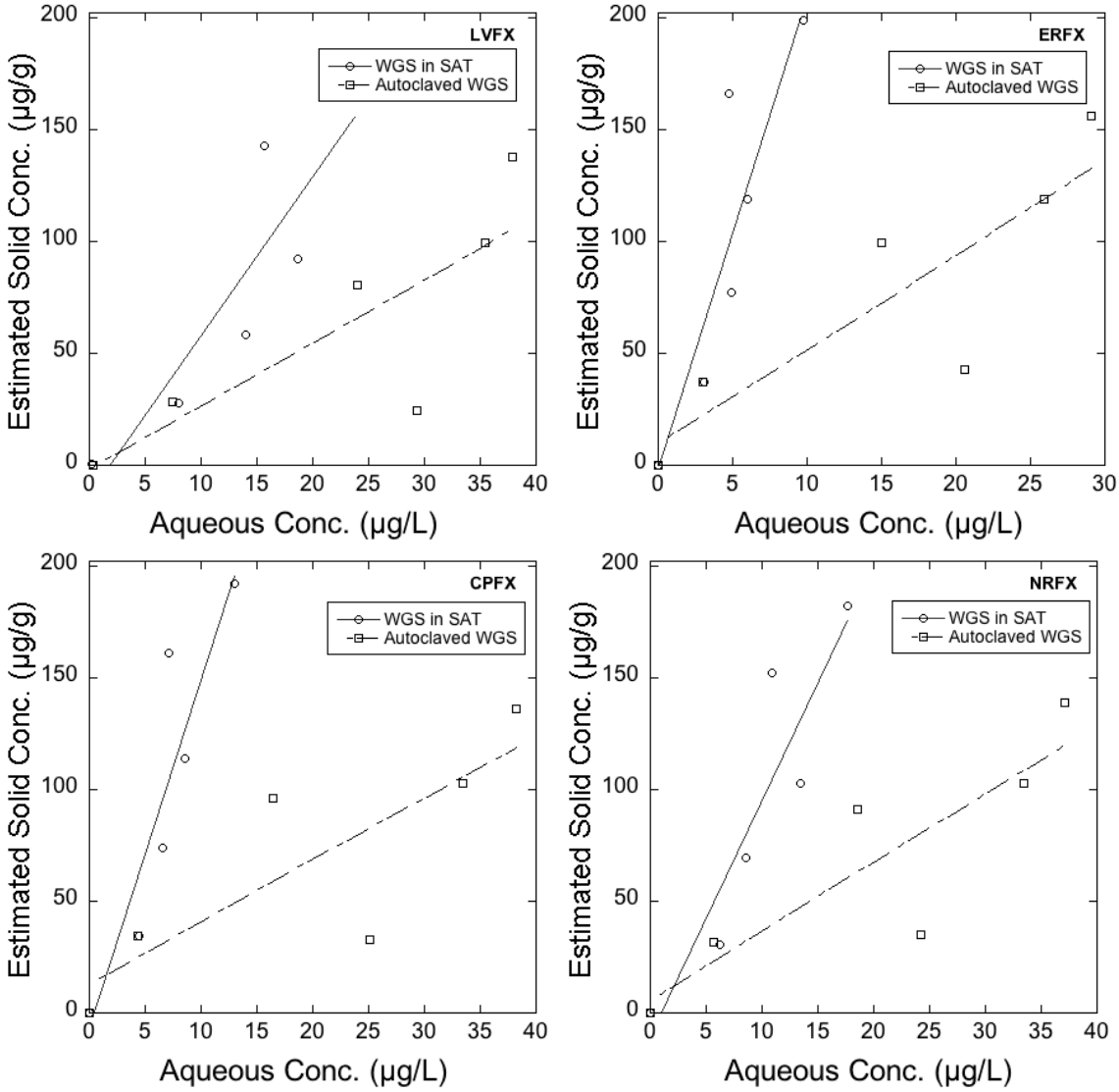

Figure 3.14 –Sorption isotherms of LVFX, ERFX, CPFX, and NRFX for WGS in SAT and autoclaved WGS.

Table 3.11 –Sorption coefficients of LVFX, ERFX, CPFX, and NRFX in WGS in SAT and autoclaved WGS.

Compounds	K_d(L/kg) of WGS in SAT WGS	K_d(L/kg) of autoclaved WGS
LVFX	5.163±2.297	2.479±1.345
ERFX	17.185±10.337	5.283±3.841

NRFX	9.159±2.796	5.061±3.203
CPFX	11.947±6.322	4.150±2.188

3.3.4. Biodegradation kinetics of PPCPs during SAT and AS system

As discussed above, biodegradation plays an important role in the removals of micropollutants in SAT. To evaluate the characteristics of biodegradation in SAT, kinetic experiments of biodegradable PPCPs were performed by soil batch-treatment experiments, and the biodegradation rates in soil batch-treatment experiments were compared with those in SBR.

For FNP, NPX, KTP, IBP, CFB, GFB, BZF, and FSM, because their pK_a and low K_{oc} value, they are all negatively charged at neutral pH, and the sorption to sludge and soil is not considered in this experiment [29-32]. Furthermore, their biodegradabilities were proven in the previous experiment. Thus, they are ideal target compounds to evaluate the biodegradation characteristics in SAT as well as AS treatment. In addition, for kinetics evaluation, first order kinetics model was chosen to describe the PPCP removal profiles in both systems. To include the differences of microbial population in SAT and AS treatment, the rate constants were calculated by the following equations:

$$\frac{dC}{dt} = -K_A C$$

$$K_{A,X} = \frac{K_A}{X}$$

Where C is the concentration of target PPCPs at each sampling time (ng/L), t is the contact time in each reactor (day), K_A is the first order rate constant (day^{-1}), X is the biomass calculated by phospholipid concentration (mmol/L), and $K_{A,X}$ is the rate constant with amount of biomass, respectively (L/ (mmol•day)).

According to the results in Tables 3.13 and 3.14, first-order kinetics were fit for the removals of most of target PPCPs in both processes (except FNP in SBR ($r^2 < 0.8$)). In SBR (Figure 3.15), FNP, KTP, IBP, and BZF were removed with half-lives less than 4 h, and NPX, CFB, GFB and FSM were removed with half-lives above 6 h. In soil batch-treatment experiments (Figure 3.15), the half-lives of selected PPCPs were above 50 h (Table 3.13), and FNP, IBP, and CFB was removed faster (half-lives <100 h) than other selected PPCPs. In addition, the kinetic constants (K_A) in SBR were higher than those in soil batch-treatment experiments.

Table 3.12 –Reaction rate constants in SBR.

Compounds	K_A (day^{-1})	r^2	$K_{A,X}$(L/(mmol•day))	Half lives (h)

FNP	0.39	0.77	10.22	1.77
NPX	0.04	0.86	0.96	18.80
KTP	0.22	0.93	5.61	3.22
IBP	0.43	0.91	11.16	1.62
BZF	0.64	0.94	16.76	1.08
CFB	0.04	0.94	1.08	16.79
GFB	0.05	0.99	1.31	13.78
FSM	0.10	0.99	2.61	6.91

Table 3.13 –Reaction rate constants in batch soil-treatment experiment.

Compounds	K_A (day^{-1})	r^2	$K_{A,X}$(L/(mmol•day))	Half lives (h)
FNP	0.014	0.97	2.85	50.69
NPX	0.004	0.92	0.89	161.60
KTP	0.003	0.94	0.68	213.46
IBP	0.013	0.97	2.71	53.32
BZF	0.004	0.96	0.75	191.70
CFB	0.008	0.88	1.72	84.05
GFB	0.004	0.78	0.72	199.67
FSM	0.005	0.96	1.05	136.98

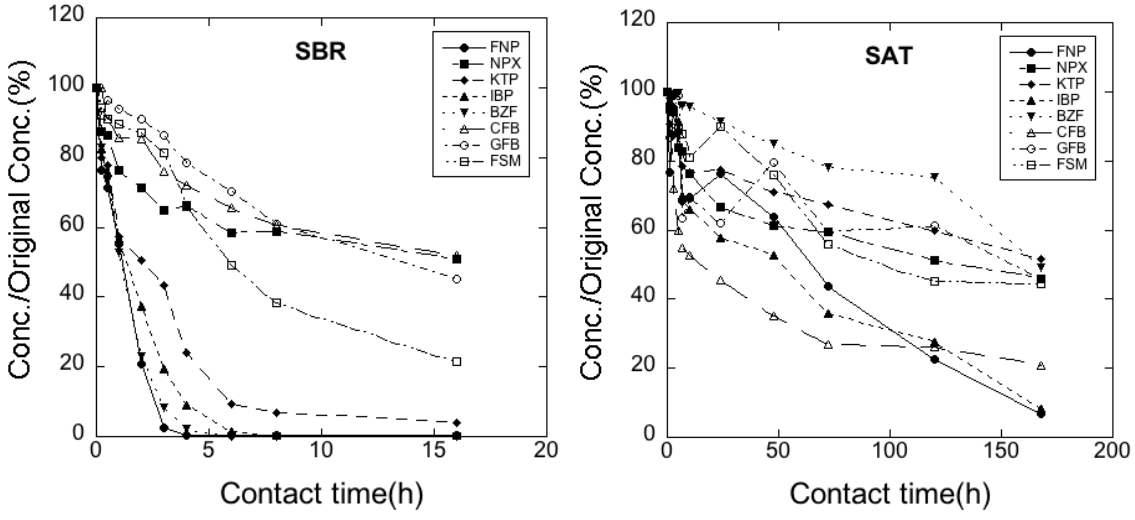

Figure 3.15 –PPCP profiles in SBR and soil batch-treatment experiment (simulation of SAT).

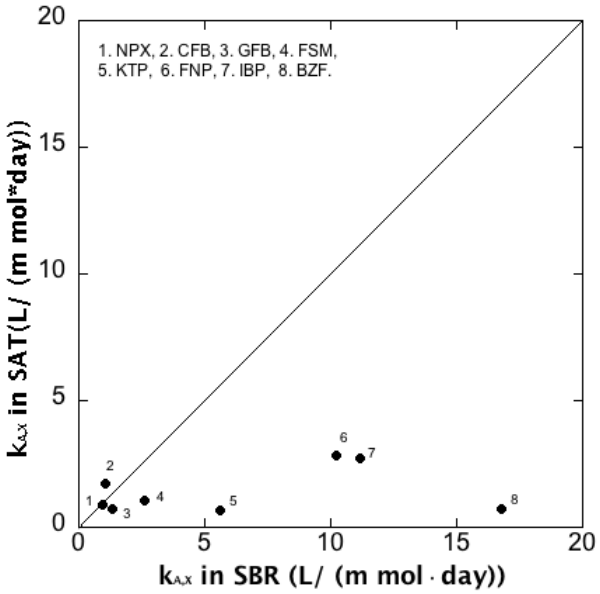

Figure 3.16 –Rate constants of target PPCPs in SBR and soil batch-treatment experiment.

The biomass concentrations of WGS in SAT and SBR were 48.01 nmol/g and 38.4 nmol/mL, respectively. The influence of biomass on biodegradation rate was evaluated by $K_{A,X}$ (Figure 3.16). The $K_{A,X}$ value of NPX, CFB, GFB and FSM were 0.96, 1.08, 1.31, and 2.61 L/(mmol•day) in SBR as well as 0.89, 1.72, 0.72 and 1.05 L/(mmol•day) in batch soil-treatment experiments, respectively. Furthermore, the $K_{A,X}$ values of BZF, IBP and FNP in SBR were 16.76, 11.16 and 10.22 L/(mmol•day), respectively, and those in batches were 0.75, 2.71 and 2.85 L/(mmol•day), respectively, which were much lower than these in SBR. In Figure 3.16, for selected PPCPs, higher $K_{A,X}$ values were obtained in SBR than in batch soil-treatment experiments.

3.3.5. Comparative study on the removals of PPCPs in SAT and AS treatment

In the previous part, the influences of biodegradation and sorption on the removals of PPCPs in SAT were evaluated. In this part, the biodegradation between AS treatment and SAT were compared by the removals of PPCPs in SBR and SAT column. In SBR, HRT of 8 h and SRT of 40 days were considered sufficient for the sorption on PPCPs according to the previous studies [6, 33, 34]. These indicated extending contact time in AS treatment cannot enhance the sorption obviously. In this study, because A_2O water and SBR sludge were sampled from the target WWTP, so SBR with treated A_2O water could be ideal to evaluate enhanced biodegradation in AS treatment. The removal efficiencies of target PPCPs were shown in Figure 3.17.

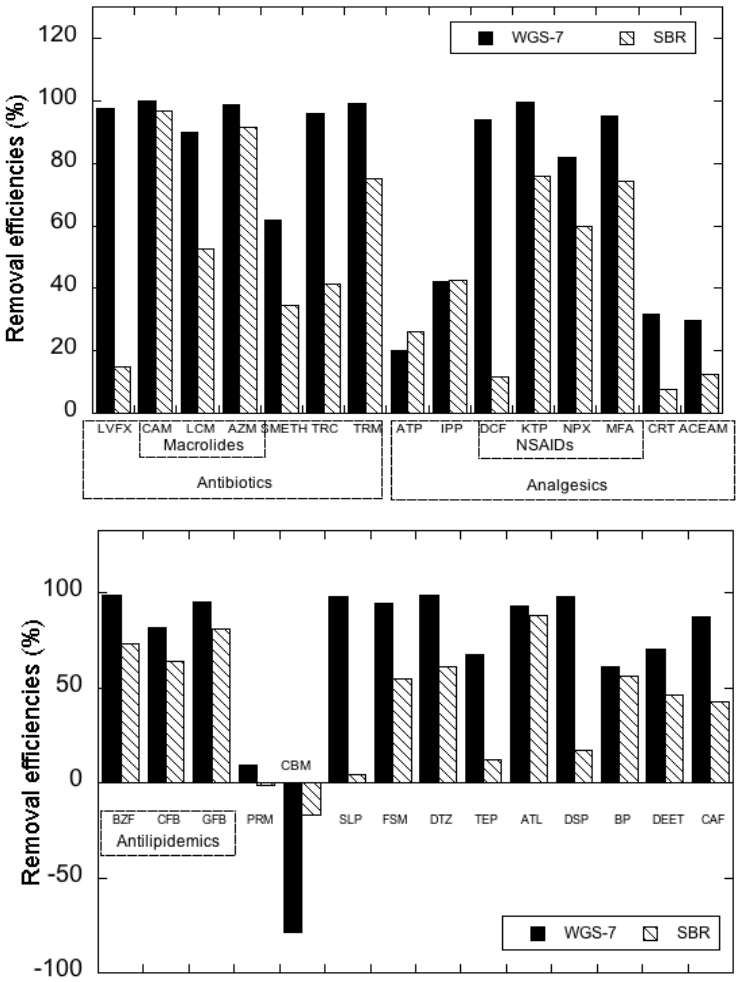

Figure 3.17 –PPCP removal efficiencies in SAT columns (WGS-7) and SBR.

As was mentioned in 3.4.2, CAM, LCM, and AZM (macrolide PPCPs) were removed effectively in SAT. In SBR, they were eliminated by 96.6%, 52.5% and 91.5%, respectively. Furthermore, TRM was removed effectively both SAT and SBR. The removal efficiencies of KTP, NPX, MFA, BZF, CFB and GFB were 75.9%, 59.8%, 74.2%, 73.1%, 64.1%, and 80.8% in SBR, respectively. The removal efficiencies in SBR were less than those in SAT. However, SLP was removed effectively in SAT (98.5%), but not in SBR (4.7%). The removal efficiencies of DCF were 94.1% and 11.7% in SAT and SBR, respectively. For CAF, its removal efficiencies in SAT and SBR were 87.2% and 42.8%, respectively. As presented above, amide PPCPs could not be removed effectively in SAT. According to Figure 3.17, amide PPCPs were not removed effectively in SBR, either. The concentration of CBM increased by 78.8% in SAT, compared with 17.2% in SBR.

3.4. Discussion

3.4.1. Occurrence of PPCPs in wastewater effluent

In order to understand the limitation of A_2O process on the control of micropollutants, the occurrence of PPCPs in A_2O water was evaluated. According to the results above, 29 PPCPs were detected frequently in the effluent from A_2O process, and the total concentration of selected PPCPs exceeded 5000 ng/L. Okuda et al. [35] reported that total concentration of 66 PPCPs in the wastewater effluents was up to approximately 10000 ng/L. These indicated that AS treatment has limited ability of removing PPCPs.

Antibiotics and analgesics were the major PPCPs among the target PPCPs.. Referring to the works of Tootchi [11], the concentration of PPCPs above 20 ng/L was considered as high concentration in the aquifer treatment. CRT was detected at the highest concentration (806 ng/L on average) in A_2O water of the target WWTP. Narumiya et al. [36] and Nakada et al. [37] reported that CRT was detected at relatively high concentration (1240 ng/L and 921 ng/L, respectively) in the effluents of several WWTPs, and its removal efficiency was low in WWTPs. For antibiotics, CAM, AZM, LVFX and CPFX were observed at relatively high concentrations (23-769 ng/L) in this study. Several studies also reported that antibiotics are present in the effluent of WWTPs at relatively high concentrations: Yasojima et al. [38] reported that LVFX, CAM and AZM were detected at concentrations of 552, 647 and 260 ng/L in the influent, respectively, and their removal efficiencies were 42%, 43% and 49%, respectively. Narumiya et al. [36] detected LVFX at an average concentration of 66 ng/L in the effluents of five WWTPs. For analgesics, KTP, DCF, MFA, ACEAM and NPX were dominant (639, 80, 75, 17, and 34 ng/L respectively). Tixier et al. [39] reported that CFB, DCF, and KTP were detected at 60, 990, and 180 ng/L in the effluent of a WWTP, respectively. In this study, IBP was not detected in A_2O water. Kobayashi et al. [40] and Narumiya et al. [36] mentioned that IBP was detected at a relatively high concentration in influent, but not detected in effluent. This is attributed to the biodegradation of IBP in treatment [37, 41, 42]. For other PPCPs, BZF, SLP, TEP, DEET, CAF, DSP and FSM were observed at high concentrations, which was in agreement with relatively high concentration detected in the effluents of WWTPs in other works [43-45].

As mentioned in Chapter 2, PPCPs cover a wide range of physicochemical properties and complex chemical structures. From the perspective of PPCP concentrations in A_2O water, PPCPs were also practical as the indicators of micropollutants in wastewater reclamation.

3.4.2. Removal mechanisms of PPCPs in SAT

3.4.2.1. Sorption and ionic interaction in SAT

Sorption is important for the removals of micropollutants in soil [46]. In this study, LVFX (K_{oc} value = 44143 mL/g) was removed highly effectively in both sterilized and non-sterilized columns. In soil batch-treatment experiment, four fluoroquinolones (LVFX, ERFX, CPFX, and NRFX) were also removed effectively in autoclaved WGS. For CPFX,

ERFX and NRFX, their K_{oc} values range from 15800-61000 mL/g, and their high removals in soil were reported [47-49]. Golet et al. [50] reported long-term persistence and limited mobility of fluoroquinolones in soils. These suggested that high K_{oc} values contributed to strong sorption in SAT. Khan et al. [46] and Jury et al. [51] mentioned that higher K_{oc} value contributed to stronger immobility of compounds, and compounds with K_{oc} value of more than 5000 mL/g were expected to be immobile in soil. For micropollutants with high K_{oc}, sorption was important for their removals in SAT, and they tend to accumulate in SAT.

For other antibiotics (i.e., CAM, LCM, and AZM), effective removals in the sterilized column indicated that abiotic degradation contributed to their removals. The K_{oc} values of CAM (150 mL/g), LCM (69 mL/g), and AZM (3100 mL/g) were much lower than those of fluoroquinolones, and hydrophobic interaction cannot explain their removals in the sterilized WGS column. However, ionic interaction was possible for their removals in SAT, because pK_a values of CAM (8.99), LCM (7.60), and AZM (8.74) range from 7.60 to 8.99, which were above the pH in SAT columns. Thiele-Bruhn et al. [52] and Lützhøft et al. [53] proposed that the sorption of antibiotics is strongly influenced by the pH of the media (soil) and ionic interaction contributed to the adsorption of antibiotics. Ionic interactions were significant for the removals of these PPCPs in soil [4]. For macrolides (i.e., CAM, LCM, and AZM), The ion-exchange interaction between positively-charge group (-$NH(CH_3)_2$) and the cations adhering to the surface of soil was expected [54, 55]. Sibley et al. [56] suggested that the sorption of CAM in soil was attributed to the electrostatic interactions between the CAM^+ tertiary amine and soil. Furthermore, for OTC, another antibiotic with low K_{oc}, its adsorption capacities improved with the enhancement of cation exchange interaction according to the previous studies [55, 57, 58]. For other PPCPs in this study, the removals of SLP (K_{oc}=13.85 mL/g, pK_a=8.99), DTZ (K_{oc}=197.8 mL/g, pK_a=8.90), ATL (pK_a=9.6), and DSP (pK_a=8.36) were also effective because of ionic interaction.

For micropollutants with high K_{oc}, sorption contributed to their removals in SAT. In addition, micropollutants with pK_a above soil pH were removed by ionic interaction in SAT. For the sorption in SAT, a limited operating time of SAT was expected because the sorption capacity was exhausted. However, in reality, the sorption processes can last for a long time. For example, the excellent and stable removals of micropollutants were expected in the Dan Region Project after 25 years of operation [59]. In the pilot plant reactors in this study, the performances on PPCP removals were stable after 3 years of operation. These were attributed to the large soil volume participating in the sorption [59], and adaptable biodegradation in SAT as discussed in 3.4.2.3 and 3.4.3.2.

3.4.2.2. The influence of biomass on sorption in SAT

In long-term SAT operation, biological communities grew in SAT, and the existence of biomass contributed to the sorption of micropollutants in SAT [5]. In this section, soil batch-treatment experiments using autoclaved WGS and WGS in SAT were operated to evaluate the influence of biomass on the sorption in SAT, and fluoroquinolones were selected as target compounds. According to the results, the values of K_d of fluoroquinolones in WGS in SAT were higher than those in autoclaved WGS

(increased with the range of 0.80 and 2.25 times). For example, the sorption coefficients (K_d) of LVFX were 2.479 L/kg in autoclaved WGS and 5.163 L/kg in WGS of SAT. This study indicated that the sorption of antibiotics was enhanced by the existence of biomass, and SAT using secondary effluent was suitable for the enhancement of sorption. Wang et al. [60] and Wu et al. [61] indicated that soil amended with biosolids enhance the sorption of herbicide terbuthylazine, TRC and triclocarban. These indicated the existence of biomass in SAT has positive influence on the sorption of micropollutants.

In this study, WGS in SAT showed a higher value of K_d for each fluoroquinolone than autoclaved WGS. The value of K_d was decided by the K_{oc} and f_{oc} (the mass fraction of soil organic carbon content). K_{oc} is decided by the characteristics of compounds. High K_d value in WGS in SAT was primarily attributed to the increase of SOM by feeding A_2O water. Lin et al. [62] mentioned that SOM increased from 0.11% in the pristine soil to ~0.8% in the 0-0.15 m soil layer with long-term operation in SAT. Thus, A_2O water with certain bacteria not only increases the biological activities in SAT (discussed on BIOLOG analysis in the following chapter), but also has a positive influence on the sorption in SAT.

3.4.2.3. Biodegradation in SAT

Biodegradation was important for the removals of micropollutants as well as sorption. For KTP, its K_{oc} value was 229-341 mL/g and its pK_a value was 4.45, so sorption and ionic interaction are not expected for its removal in SAT. In this study, the removal efficiency of KTP in the non-sterilized columns was higher than that in the sterilized columns. These indicated that the removal of KTP in SAT was attributed to the biodegradation in SAT. Similarly, NPX was also removed by biodegradation in SAT. Monteiro and Boxall [63] reported that NPX was degraded rapidly in agricultural soils, and Macro-Urrea et al. [64] found the white-rot fungus can degrade NPX. For other profens, Drewes et al. [65] and Onesios et al. [66] reported that IBP and FNP were easily biodegradable. These indicated that profens were biodegradable in SAT. Removals of BZF and GFB were also mainly attributed to the biodegradation. Tiehm et al. [67] and Fang et al. [68] mentioned that BZF and GFB were biodegradable in aquifer recharge and soil. MFA was removed efficiently by biodegradation, and MFA was removed moderately in the sterilized columns in this study. This could be attributed to the sorption in soil, which was also mentioned in the previous studies [42, 69, 70]. In addition, CAF and FSM were also mainly removed by biodegradation in SAT. From the perspectives of functional groups, biodegradable PPCPs in SAT expect CAF contained carboxyl groups. Bertelkamp et al. [71] demonstrated that carboxyl groups increased the biodegradation rates of micropollutants. These indicated that carboxyl groups improved the biodegradability of micropollutants in SAT.

For CFB, Buser et al.[72] assumed that CFB was more stable than DCF and IBP, and the biodegradation of CFB contributed its 50% loss in WWTP. From the perspective of chemical structures, CFB contained halogen in its chemical structure, which can decrease the biodegradability of compounds [71]. In this study, CFB was removed effectively in the SAT column with an HRT of 7 days, indicating that CFB can be removed by biodegradation in SAT with a relatively long HRT. With long-term operation,

the biodegradation of CFB was expected in SAT under aerobic and anoxic conditions [2]. The biodegradation rate of biodegradable PPCPs containing carboxylic acid in AS treatment and SAT will be discussed in 3.4.3 with respect to functional groups. Furthermore, the biodegradations of micropollutants (i.e., isoproturon, polycyclic aromatic hydrocarbon (PAH), and PCB) were formed in soil [73, 74]. This could be attributed to the long HRT in SAT [75]. The influence of HRT on the removals of PPCPs would be discussed in 4.4.3.

In summary, KTP, NPX, IBP, FNP, antilipidemics, FSM and CAF were biodegradable in SAT, and most biodegradable PPCPs except CAF contained carboxyl groups. Thus, the presence/absence of carboxyl group in chemical structure is important to predict the biodegradability of micropollutants in SAT.

3.4.2.4. Persistent compounds in SAT

According to the results, SAT removed most PPCPs effectively, but it still had the limitation on controlling some PPCPs such as amide PPCPs. CBM was resistant in both the sterilized and non-sterilized columns. More specifically, CBM increased in the non-sterilized columns. Yonetani [76] mentioned that CBM metabolites in the A_2O water could be deconjugated into CBM. Yang et al. [77] reported that ERM and CBM were resistant to biological treatment. The CBM molecule is uncharged and polar, and its interactions with soils and minerals was weak [32, 77]. Thus, the sorption of CBM to minerals, soil components or sediments was not expected. The removal of CRT was poor in both sterilized and non-sterilized columns. Shinohara et al. [78] and Kasprzyk-Hordern et al. [79] demonstrated that the removal efficiencies of CRT were very low in soil infiltration. These results showed that amide PPCPs were resistant in SAT, and the existence of amide functional groups suppressed the biodegradability of compounds in SAT. Amide groups contain carbonyl group and amino group, and conjugated system is expected in amide groups because of the electron pair in amino group and π-electron in carbonyl group, resulting in the decease of density of electron cloud. The aromatic ring structure further decrease the density of electron cloud because of its conjugated system with nitrogen atom in amide groups [80]. These explained that poor biodegradability of amide PPCPs in SAT and AS treatment because of the existence of amide groups and benzene ring.

Though amide PPCPs were persistent in SAT, they were removed effectively by other treatments. For example, CBM was removed rapidly by ozonation and advanced oxidation process (AOP, i.e., UV/H_2O_2) [81, 82]. Okuda et al. [35] reported that ozonation process significantly decrease SLP, CRT, DSP and SMETH in secondary effluent. In order to improve the removals of micropollutants with amide functional groups, advanced treatment processes should be considered.

3.4.3. Comparison between SAT and AS treatment

3.4.3.1. Kinetics on biodegradable PPCPs between SAT and AS treatment

Carboxylic compounds were biodegradable according to the results in this study, but different carboxylic compounds (FNP, NPX, KTP, IBP, CFB, GFB, BZF, and FSM)

showed different extent of biodegradabilities in SAT and AS treatment. Biodegradation rates of selected PPCPs in SBR were in the following order: BZF > IBP > FNP > KTP > FSM > GFB > CFB > NPX. In SBR, biodegradable PPCPs could be divided into two groups (Figure 3.15): 1) The half-lives of GFB, FSM, CFB and NPX were above 6 h; 2) The half-lives of FNP, KTP, IBP and BZF were less than 6 h. Wise [83] mentioned that hydroxyl and carboxyl groups on aromatic rings increased biodegradabilities and halogen, nitro, and sulfonate functional groups on aromatic rings decreased biodegradabilities. From the perspective of functional groups, halogens contributed to the chemical stability, resulting in their suppression of biodegradation [84]. PAHs were more persistent than other organic matter in activated sludge systems [85], and complicated aromatic ring structures can suppress the biodegradability of compounds [86, 87]. FSM and CFB possess halogens, and NPX possesses naphthyl, which was the derivative of PAH, leading to their less active biodegradabilities in SBR. For the easily biodegradable PPCPs (BZF, FNP, KTP, and IBP), FNP, KTP and IBP have the common part of 2-arylpropionic acids, a carboxylic acid. For BZF, halogen suppress its biodegradability, but the carboxyl group and straight chain structure increased its biodegradability[88]. Thus, the biodegradation rate of BZF was faster in SBR than other halogenated acids.

In the soil batch-treatment experiment, BZF, IBP, FNP, KTP, FSM, GFB, CFB, and NPX degraded steadily, and biodegradation rates of selected PPCPs in this experiment were in the following order: FNP > IBP > CFB > FSM > NPX > BZF > GFB > KTP. In soil batch-treatment experiment, selected PPCPs were removed effectively as SBR, but the order of biodegradability was different. With long contact time, the biological communities in soil batch-treatment experiment could be different from SBR [8]. The difference of biological communities between SBR and different packing materials in SAT will be further discussed in 4.4.1.

IBP, KTP, FNP and BZF are more biodegradable than NPX, FSM, CFB and GFB in AS treatment. For the biodegradation in AS treatment, cometabolic biodegradation is expected for the micropollutants including IBP, KTP, and BZF [89-92]. As well as AS treatment, the concentration of carbon source such as DOC enhance the cometabolic biodegradation in other biological treatments including SAT [93, 94], but the order of biodegradation for selected PPCPs are different in SAT. This could be attributed to lower DOC in SAT influent than that in influent of SBR. In addition, the biodegradation rates with certain biomass ($K_{A,X}$) of KTP, FNP, IBP, and BZF in SAT were lower than those in SBR. However, the $K_{A,X}$ values of NPX, CFB, GFB, and FSM in SAT were similar to those in SBR. The HRT in SAT is longer than that in SBR. With long-term operation, adaptable biological communities for the biodegradation of PAHs and PCBs were expected in soil [95, 96]. Diercxsens et al.[97] demonstrated that adapted biodegradation for micropollutants which were not completely degraded in AS treatment, was expected in soil with continuous discharge of sewage effluent. Margesin et al.[98] mentioned that build-up of microbial communities was expected in soil with feeding contaminants. In addition, SRT played an important role in the biodegradation in AS treatment [99]: Wever et al. [100] reported that MBR treatment obtaining longer SRT than AS treatment could significantly enhance the removals of 1,6- and 2,7-naphthalene disulfonate (NDSA) and benzothiazole-2-sulfonate. Göbel et al. [101] reported that TRM was removed by above 90% at a SRT of 60–80 days. Similarly, SAT obtained a much longer SRT than AS

treatment. Thus, for NPX, CFB, GFB, and FSM, though their cometabolic biodegradation was not expected because of low DOC concentration as discussed above, adaptable biodegradation in SAT could be attributed to the long-term operation and long HRT in SAT.

In Figure 3.16, for selected PPCPs, higher $K_{A,X}$ values were obtained for SBR than soil batch-treatment experiments. This could be attributed to that easy transportation of compounds to microorganisms because of good mixture of activated sludge and compounds in AS treatment. The contact time in SBR (8 h) was shorter than in soil batch-treatment experiments (7 days), so the removal characteristics should be evaluated combining with contact time. In Table 3.14, the values of $K_{A,X}\times t$ between SBR and soil batch-treatment experiments were compared. $K_{A,X}\times t$ in batch soil-treatment experiment (SAT) were more sufficient than those in SBR. This indicated that SAT could further degrade micropollutants with long contact time.

Table 3.14 –Comparison of $K_{A,X}\times t$ between SAT and SBR.

Compounds	$K_{A,X}\times t$ in SAT (L/(mmol))	$K_{A,X}\times t$ in SBR(L/(mmol))
FNP	19.94	3.41
NPX	6.26	0.32
KTP	4.74	1.87
IBP	18.96	3.72
BZF	5.27	5.59
CFB	12.03	0.36
GFB	5.06	0.47
FSM	7.38	0.87

3.4.3.2. Differences of biodegradation between SAT and AS treatment

In this part, the differences of biodegradation between SAT and AS treatment are discussed. At first, For the easily absorbable PPCPs in SAT (i.e., LVFX), LVFX was removed poorly in SBR. Similarly, SLP was also removed less in SBR than that in SAT columns. In activated sludge system, PPCPs are mainly removed by sorption and biodegradation [102, 103]. LVFX and SLP were mainly removed by sorption in soil of SAT columns as discussed above. Okuda et al.[6] observed that SLP was detected at relatively high concentration in the secondary effluent of WWTPs. These indicated that effective biodegradation of LVFX and SLP were not expected in AS treatment as well as in SAT.

For the PPCPs removed by ionic interaction (i.e., CAM and ACM), In SBR, CAM, and ACM were removed effectively because of biodegradation. Yang et al. [77], Yasojima et al. [38], and Göbel et al. [4] mentioned that macrolide PPCPs were readily removed in AS treatment. Göbel et al. [4] reported that CAM and ACM were detected in activated sludge samples, but only 5% to 7% of CAM in influent was transformed into

the activated sludge solid based on the analysis of mass balance of CAM in activated sludge. This indicated that their sorption was not expected in SBR. In addition, Göbel et al. [101] mentioned that CAM and dehydro-ERM were removed effectively with a long SRT and continuous load of PPCPs leads to an increased biodiversity of the active biomass, resulting in a broader range of degradation pathways. Thus, in this experiment, together with the long SRT (40 days in SBR), effective removal of CAM and ACM in SBR could be attributed to the biodegradation. For LCM, its removal was efficient in SAT columns, but the removal of LCM in SBR was moderately efficient. This suggested that the biodegradation of LCM was weaker than other macrolide PPCPs. From the perspective of chemical structure, LCM has an amide functional group, which can suppress the biodegradability of compounds. TRM was removed at 75.1% in SBR. However, literature showed a large variation on the removal of TRM (from -40% to 70%)[79, 101, 104, 105]. Efficient removals of TRM have been reported for TRM in nitrifying sludge with longer sludge ages than in AS treatment [104]. Similarly to macrolide PPCPs, enhanced biodegradation was expected for the removal of TRM in SBR by extending contact time and SRT. Compared with in SAT, enhanced biodegradation by extending contact time was expected in AS treatment instead of ionic interaction.

For the biodegradable PPCPs in SAT, DCF was removed poorly in SBR in comparison with SAT, which was consistent with the poor removal of DCF in AS treatment [106, 107]. This was attributed to weak sorption and biodegradation of DCF in activated sludge [106]. For the effective removal of DCF in SAT, while sorption was not expected in SAT as discussed in 3.4.2, biodegradation of DCF in soil can be enhanced by long contact time [33-35, 108, 109]. From the perspective of chemical structure, DCF is a halogenated acid containing two halogens, and this suppressed its biodegradation in AS treatment. In SAT, the biodegradation of DCF was formed because of a long HRT, which was similar to the biodegradation of CFB, FSM, and BZF as discussed in 3.4.3.1. In the previous studies, CAF was removed effectively by more than 90% [110, 111]. In SBR, removal of CAF was less effective (42.8%) than that in AS treatment by the previous studies because of sorption equilibrium of biomass and A_2O water. CAF cannot be degraded rapidly as other biodegradable PPCPs in activated sludge treatment [112]. In addition, the removal of CAF in SAT column was more effective (87.2%) than in SBR. With long contact time, the biodegradation of CAF was adapted in SAT [113]. Thus, CAF was moderately removable PPCPs in SBR, and its biodegradation can be adapted in SAT. For other moderately removable PPCPs in SBR such as FSM and DEET, similar enhancement on biodegradation was expected in SAT. For biodegradable PPCPs, as discussed in Part 3.4.2 and 3.4.3, KTP, NPX, MFA, BZF, CFB, and GFB were also biodegradable in SBR, which was in agreement with the works by other researches [110, 111, 114, 115]. Their removal efficiencies were higher in SAT than in SBR, which could be attributed to the adapted biodegradation and long contact time. From the perspective of biodegradation characteristics, SAT has a wider biodegradation range than AS treatment.

For amide PPCPs, their removals were poor in SBR as well as in SAT. Clara et al. [116] also mentioned that amide PPCPs are neither subjected to biodegradation nor to adsorption processes during wastewater treatment.

As summarized in Table 3.15, SAT showed highly efficient and efficient removals on antibiotics and carboxylic PPCPs. In SBR, CAM, AZM, GFB, and ATL were removed efficiently. In summary, SAT has a wider biodegradation range than AS treatment. More specifically, SAT has the advantages on the sorption of antibiotics and enhanced biodegradation of moderately removable compounds in AS treatment.

Table 3.15 –Comparison of removal efficiencies of PPCPs in SAT and SBR.

Categories of removal efficiencies	WGS-7 column	SBR
Highly efficient (>90%)	LVFX, CAM, LCM, AZM, TRC, TRM, DCF, KTP, MFA, BZF, GFB, SLP, FSM, DTZ, ATL, DSP,	CAM, AZM,
Efficient (80-90%)	NPX, CFB, CAF	GFB, ATL
Moderate (50-80%)	SMETH, TEP, BP, DEET,	LCM, TRM, KTP, NPX, MFA, BZF, CFB, FSM, DTZ, BP
Poor (<50%)	ATP, IPP, CRT, ACEAM, PRM,CBM	LVFX, SMETH, TRC, ATP, IPP, DCF, CRT, ACEAM, PRM, CBM, SLP, TEP, DSP, DEET, CAF

3.5. Summary

Firstly, soil in SAT showed strong sorption on micropollutants possessing high K_{oc}. The existence of biological communities (i.e., biomass) enhanced the sorption in SAT. In addition, ionic interaction in SAT contributed to the removal of compounds having the values of pK_a above the soil pH.

Secondly, biodegradation played an important role in the removals of micropollutants in both AS treatment and SAT. Carboxyl groups increase the biodegradability, and amide functional groups decrease the biodegradability in both treatments. Biodegradation of halogenated acid and PAH was less efficient in AS treatment than in SAT. AS treatment degraded the biodegradable compounds rapidly, but SAT was effective for a wider range of compounds.

Overall, for the control of micropollutants, AS treatment degraded easily biodegradable compounds rapidly, and SAT has the advantage on the sorption of

compounds as well as the further biodegradation on moderately biodegradable compounds. Thus, the combination of AS treatment and SAT could be an effective choice of controlling micropollutants for improving water quality.

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Chapter 4 Effects of operating conditions on the removals of PPCPs in SAT

4.1. Introduction

As discussed in Chapter 3, sorption and biodegradation were both important for the removals of micropollutants in SAT. From the perspective of practical use, operating conditions in SAT affected the performance of SAT on the removals of pollutants, for example, organic carbon, the presence of specific microorganisms, DO conditions, temperature, pH, and moisture content influenced the sorption and biodegradation of micropollutants in SAT [1-3]. However, the information on the removals of PPCPs in SAT under different conditions is little. Furthermore, enhanced biodegradation was expected in SAT because of a longer HRT than AS treatment, so the effect of HRT on biodegradation should not be ignored in SAT, and a suitable HRT for SAT should be determined.

As presented in Chapter 2, the presence of vadose (i.e., unsaturated) zone has a major impact on SAT performance. For example, removals of organic matters varied with the depth of the vadose level in SAT [4]. In Chapter 3, the contribution of biodegradation on the removals of PPCPs in SAT was well understood, but the influence of aerobic and anaerobic condition on the biodegradation in SAT was not clear. From the perspective of practical use, the layer depth of saturated layer and vadose layer is as important as HRT for the site design of SAT[5]. Thus, the influence of saturated and vadose conditions on PPCPs removals in SAT should be evaluated to understand the influence of saturated condition on the removals of micropollutants.

For evaluating the influence of operating conditions on the removals of micropollutants in SAT, as presented in Chapter 3, PPCPs were used as the indicators of micropollutants in SAT. In this chapter, firstly, the biological communities in SAT under different conditions are compared with those in AS treatment; secondly, the influences of packing materials, HRT and vadose condition on the removals of PPCPs in SAT are evaluated. Finally, practical advises on the removals of micropollutants in SAT are provided.

4.2. Materials and methods

Microbial substrate metabolic patterns (BIOLOG) were used for evaluating differences of biodegradation in SAT columns under different conditions. In SAT column experiments, firstly, columns packed with WGS, sand and TS were operated to evaluate the influences of packing materials on the sorption and biodegradation in SAT because they have different properties such as CEC, SOM and specific area as described in Table 3.1. Secondly, Mansell et al.[6] mentioned that biodegradable organic carbon was effectively removed typically in 5 days. Thus, the column experiments for the study of HRT were divided into two parts (short-term (hr scale) and long-term (day scale)) to understand the influence of HRT from operation. HRTs of 3.5, 7, and 30 days in sand columns and HRTs of 7, 30, and 180 days in WGS columns were evaluated to understand the influences of long-term operation (day-scale) in SAT. Based on the results in the first part, the HRTs of 7 days, 1 day, 8 hrs and 4 hrs were compared to understand the removal trend of PPCPs in the short-term operation (hr-scale). Thirdly, columns were operated under vadose and saturated conditions to evaluate the effect of vadose condition on the removals of PPCPs in SAT.

4.2.1. Reagents

The reagents used in this chapter have been mentioned in Part 3.2.1.

4.2.2. Column experiments

4.2.2.1. Column setting

As presented in Part 3.2.2, columns under different operating conditions were operated in this study. Effluent from the WWTP mentioned in Chapter 3 was used as feed water for SAT columns. Four large lab-scale acrylic columns (TS-7, Sand-3.5, Sand-7, and Sand-Sat; i.d., 15 cm; H, 150 cm), two medium lab-scale acrylic column (WGS-1 and WGS-7; i.d., 8 cm; H, 100 cm), two small lab-scale acrylic columns (WGS-4H and WGS-8H; i.d.,5 cm; H, 40 cm), and four pilot plant-scale stainless steel reactors (Sand-30, WGS-30, WGS-180, and WGS-Sat; L, 1.5 m; W, 1.5 m; H, 3.0 m) were used. The operating conditions were summarized in **Tables 4.1 and 4.2**. Feed water for large lab-scale acrylic columns was fed by peristaltic pumps (7554-80, Thermo Fisher Scientific), and feed water for medium and small lab-scale acrylic column was fed by peristaltic pumps (SJ1220, ATTA). The schematic of lab-scale acrylic column is shown in Figure 3.1.

Feed water for pilot plant-scale stainless steel reactors was pumped into reactors by centrifugal pumps. The pilot-plant scale reactors packed with WGS consisted of the bottom layer (gravel level) and the soil layer. For the vadose condition, the water head was maintained at 20 cm below the top of the soil/sand layer. For the saturated condition, the water head was maintained at 20 cm above the top of the soil layer. The operation of sand pilot plant-scale reactor started in October 2011; and WGS pilot plant-scale reactors started in April 2012. The schematic of pilot plant-scale reactors is shown in Figure 4.1.

Table 4.1 –Operating conditions of SAT columns under saturated conditions.

Columns	Scale	Soil types	Hydraulic retention time (HRT)
Sand-Sat	Lab	Common sand	7 days
WGS-Sat	Lab	WGS	30 days

Table 4.2 –Operating conditions of SAT columns under vadose conditions.

Columns	Scale	Soil types	Hydraulic retention time (HRT)
TS-7	Lab	Toyoura standard sand	7 days
Sand-3.5	Lab	Common sand	3.5 days
Sand-7	Lab	Common sand	7 days
WGS-7	Lab	WGS	7 days
Sand-30	Pilot plant	Common sand	30 days
WGS-30	Pilot plant	WGS	30 days
WGS-180	Pilot plant	WGS	180 days
WGS-1	Lab	WGS	1 days
WGS-4H	Lab	WGS	4 hrs
WGS-8H	Lab	WGS	8 hrs

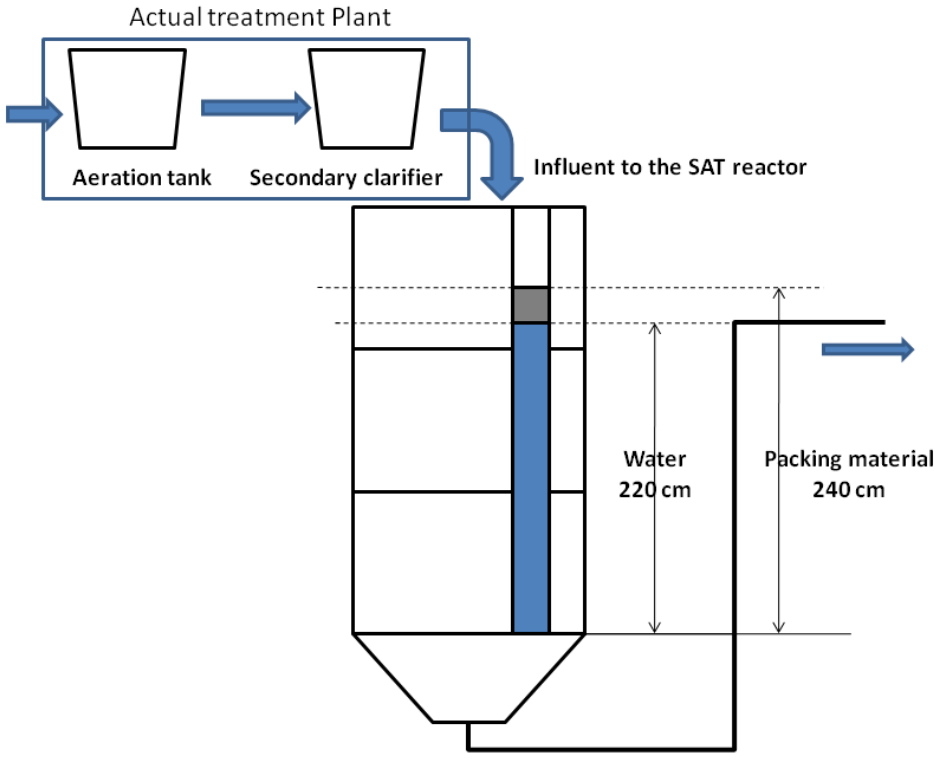


Figure 4.1 –Schematics of the SAT reactors under the vadose condition.

4.2.2.2. Sampling method

The operation of large lab-scale acrylic columns started in January 2012. The operation of the medium lab-scale acrylic columns started in April 2013. The operation of the small lab-scale acrylic columns started in July 2014. SAT effluents from large lab-scale columns and plant pilot-scale reactors were sampled seven times (October 4th, October 22th, December 2nd, and December 25th in 2013, followed by January 14th, February 6th, and February 25th in 2014). SAT effluents from small lab-scale columns were sampled three times (September 28th and October 20th in 2014, followed by January 3rd in 2015).

4.2.3. Analytical methods

Pretreatment and quantification methods for PPCPs were the same as described in Chapter 3.

4.2.4. Evaluation of microbial substrate metabolic patterns

The method for the evaluation of microbial substrate metabolic patterns was designed referring to the work by Takabe et al. [7] as described below.

4.2.4.1. Sampling scheme

Activated sludge in AS treatment (AS) was collected from the aerobic tank in the target WWTP; activated sludge in SBR (SBR) was collected from the lab-scale SBR; A_2O water (A_2O) was collected from the effluent of A_2O process in the target WWTP. Samples of Saturated WGS (S.WGS) were collected in column of WGS-Sat. Samples of New WGS (NWGS) and New sand (Nsand) were collected in the stored WGS and sand without use. Other samples were collected in appropriate columns (WGS-180, WGS-30, and Sand-30, respectively).

Table 4.3 –Samples of BIOLOG.

Samples	Sampling point	Samples	Sampling point
AS	Activated sludge in AS treatment	A_2O	A_2O water
SBR	Activated sludge in SBR	S.WGS	Saturated WGS
NWGS	Stored WGS without use	Nsand	Stored sand without use
WGS-180	WGS-180 column	WGS-30	WGS-30 column
Sand-30	Sand-30 column		

For sampling soil and sand in pilot plant-scale reactors, a liner sampler (DIK-110C, Daiki Rika Kogyo, Japan, 5 cm (D) × 30 cm (H)) was used in the surface of SAT reactors (center area). The liner sampler, the stainless spoon for samples collection, and sampling tubes were autoclaved before use.

4.2.4.2. BIOLOG assay with EcoPlate

EcoPlate (Biolog Inc. U.S.) was used to compare the microbial substrate metabolic patterns among nine types of samples: AS, SBR, A_2O, WGS30, WGS180, Sand30, S.WGS, NWGS, and Nsand.

AS and SBR were diluted 100 times with autoclaved sodium phosphate buffer (pH= 7.6, 60 mM), and A_2O was diluted 10 times with the same solution.

For soil and sand samples, microorganisms in the sand and soil (WGS30, WGS180, Sand30, S.WGS, NWGS, and Nsand) were dispersed from soil and sand particles with a Waring blender (Ace homogenizer AM-3, Nihonseiki, Japan). Samples were collected on August 6th and September 6th in 2015. A mixture of 10 g of soil/sand and 40 mL of the phosphate buffer was blended in the blender for 3 mins. The homogenate was centrifuged for 1 min at 1500 rpm (KUBOTA 5200, Kubota, Japan) to settle the particles. Because it is difficult to remove the soil particles from homogenate the method of Takabe et al.[7] was modified: 20 mL of the supernatant was filtrated with 1.0 μm glass fiber (GA-100, ADVANTEC); 15 mL of filtrate and 5 mL of supernatant were mixed for BIOLOG analysis. All treatments above were operated in a clean bench. Autoclaved WGS and sand were used as control samples.

For the incubation in EcoPlates, 150 μL of diluted samples (AS, SBR, and A_2O) and filtrates (WGS30, WGS180, Sand30, S.WGS, NWGS, and Nsand) were incubated in the wells of the plates. EcoPlates were incubated at 25 ^{o}C in an incubator (IS-41, Yamato, Japan). In the initial phase, the absorbances of samples were monitored by using a Powerscan-PC (DS Pharma Biomedical, Japan) at every 1.5 hrs until the value of average well color development (AWCD) [8, 9] reached 0.2 [10], then the absorbances of samples were recorded every 12 hrs for 8 days. Wet paper was set at the bottom of the incubator to maintain the humidity.

4.3. Results

4.3.1. Microbial substrate metabolic patterns

As discussed above, biodegradation played an important role in the removals of PPCPs, and SAT under different conditions may have different characters on the biodegradation of PPCPs. The differences of PPCP biodegradation between SAT and SBR were discussed in details in Chapter 3. In this section, for evaluating microbial substrate metabolic patterns in SAT reactors and AS treatment (AS, SBR, A_2O, WGS30, WGS180, Sand30, S.WGS, NWGS, and Nsand), a series of BIOLOG assays were performed.

Table 4.4 shows the normalized absorbance of each substrate in the samples, and 31 substrates were classified based on their structures in reference to the work of Salmo et al. [10]. Normalization formula is shown as follows.

$$Normalized\ data = \frac{(x - \min(x_1, x_2, \ldots\ x_n)}{(\max(x_1, x_2, \ldots x_n) - \min(x_1, x_2, \ldots x_n))}$$

Where x is the absorbance data of target substrate at AWCD of 0.2, and x_1, x_2 ... x_n was the absorbance data of each substrate.

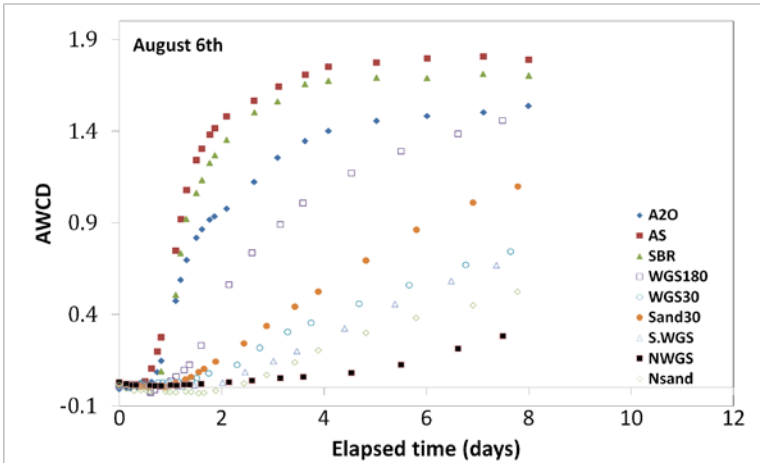
In Table 4.4, normalized values above 0.1 were marked as positive (shaded values). In the activated sludge samples (A_2O, AS, and SBR), seven carbohydrates (D-cellobiose, D-galactonic acid γ-lactone, D-mannitol, i-erythritol, N-acetyl-D-glucosamine, and β-methyl-D-glucoside), three carboxylic acids (D-glucosaminic acid, D-malic acid and γ-hydroxylbutyric acid), two amino acid (glycyl-L-glutamic acid and L-asparagine), and one polymer (tween 80) were positive. A wider range of substrates in SAT samples (WGS30, WGS180, Sand30 and S.WGS) was metabolized than those in the samples of activated sludge. For example, WGS30 and WGS180 samples showed a positive response for the degradation of four carbohydrates (D-cellobiose, D-mannitol, N-acetyl-D-glucosamine and α-D-lactose), two carboxylic acids (itaconic acid and α-ketobutyric acid), three amino acids (L-arginine, L-asparagine, and L-phenylalanine), one amine (putrescine), three polymers (glycogen, tween 40, and tween 80), phosphorylated chemicals (D,L-α-glycerol phosphate) and Esters (pyruvic acid methyl ester).

Growth rates of AWCD were evaluated as shown in Figure 4.2. The AWCD values of AS, SBR, and A_2O samples increased more rapidly than other samples, and the growth rates of AWCD ranked as follows: AS > SBR > A_2O > WGS180 > Sand30 > WGS30 > S. WGS > Nsand > NWGS. Finally, AWCD values of SAT samples were higher than those values of non-used packing materials.

The substrate metabolic patterns were similar in the samples of August 6th and September 6th samples. By using principal component analysis (PCA), the substrate metabolic patterns of samples were evaluated in the plot as shown in Figure 4.3 (Where FPC referred to the first principal component, SPC referred to the second principal component). AS, SBR and A_2O samples were located at close position in the plot, and this indicated that these samples had similar substrate metabolic patterns. The metabolic patterns exhibited by SAT samples (WGS30, WGS180, Sand30 and S.WGS) were at a midpoint of those exhibited by non-used materials (Nsand and NWGS) and activated sludge samples (AS, SBR and A_2O samples). That is, the metabolic patterns of SAT samples were different from those of activated sludge and natural soil. In addition, among SAT samples, the fact that the plot of data of WGS180 was located at different positions compared with other SAT samples. These indicate that the metabolic pattern of WGS180 was different from other SAT columns.

Table 4.4 –Normalized BIOLOG data (absorbance of each substrate) for samples

Chemical groups	Substrates	Samples								
		A2O	AS	SBR	WGS30	Sand30	WGS180	S.WGS	NWGS	Nsand
Carbohydrates	D-cellobiose	0.335	0.215	0.160	0.601	0.381	0.494	0.520	0.000	0.020
	D-galactonic acid γ-lactone	0.193	0.388	0.190	0.000	0.078	0.265	0.063	0.303	0.132
	D-mannitol	0.371	0.132	0.103	0.182	0.793	0.481	0.099	0.271	0.591
	D-xylose	0.033	0.025	0.010	0.000	0.038	0.991	0.000	0.000	0.015
	i-erythritol	0.768	0.907	1.000	0.000	0.022	0.412	0.011	0.001	0.002
	N-acetyl-D-glucosamine	0.326	0.500	0.224	0.522	1.000	0.080	0.100	0.022	0.225
	α-D-lactose	0.370	0.202	0.000	0.505	0.309	0.046	0.079	0.019	0.015
	β-methyl-D-glucoside	0.968	0.586	0.462	0.051	0.731	0.736	0.131	0.256	0.010
carboxylic acid	4-hydroxy benzoic acid	0.000	0.022	0.066	0.071	0.083	0.182	0.022	0.428	0.229
	D-glacturonic acid	0.046	0.014	0.000	0.011	0.145	0.101	0.450	0.004	0.026
	D-glucosaminic acid	0.694	0.876	0.349	0.070	0.020	0.062	0.045	0.009	0.142
	D-malic acid	1.000	1.000	0.489	0.001	0.000	0.421	0.069	0.614	0.034
	itaconic acid	0.012	0.014	0.034	0.133	0.132	0.254	0.043	0.282	0.000
	α-ketobutyric acid	0.005	0.002	0.008	0.146	0.129	0.194	0.110	0.272	0.036
	γ-hydroxylbutyric acid	0.149	0.306	0.183	0.072	0.037	0.253	0.199	0.119	0.134
	2-hydroxy benzoic acid	0.075	0.046	0.000	0.067	0.034	0.073	0.087	0.221	0.043
Amino acid	Glycyl-L-glutamic acid	0.241	0.214	0.114	0.087	0.016	0.618	0.025	0.021	0.019
	L-arginine	0.000	0.000	0.000	0.494	0.172	0.477	0.393	0.404	0.702
	L-asparagine	0.158	0.042	0.104	1.000	0.384	0.461	1.000	0.798	1.000
	L-phenylalanine	0.034	0.065	0.015	0.238	0.211	0.345	0.079	0.551	0.046
	L-serine	0.016	0.024	0.017	0.032	0.375	0.543	0.113	0.072	0.857
	L-threnine	0.034	0.061	0.000	0.063	0.023	0.005	0.036	0.046	0.020
Amines	Phenylethyl-amine	0.012	0.004	0.000	0.062	0.035	0.064	0.090	0.089	0.000
	Putrescine	0.051	0.053	0.007	0.581	0.201	0.350	0.273	0.451	0.428
Polymers	Glycogen	0.132	0.088	0.114	0.149	0.786	1.000	0.113	0.146	0.035
	Tween 40	0.063	0.020	0.002	0.224	0.383	0.593	0.537	0.810	0.434
	Tween 80	0.194	0.248	0.145	0.255	0.316	0.359	0.124	1.000	0.380
	α-cyclodextrin	0.022	0.010	0.001	0.076	0.000	0.062	0.000	0.114	0.014
Phosphorylated chemicals	D,L-α-glycerol phosphate	0.021	0.071	0.008	0.171	0.231	0.000	0.000	0.000	0.020
	Glucose-1-phosphate	0.000	0.232	0.000	0.072	0.000	0.137	0.005	0.000	0.024
Esters	Pyruvic acid methyl ester	0.010	0.048	0.050	0.895	0.349	0.155	0.443	0.691	0.651



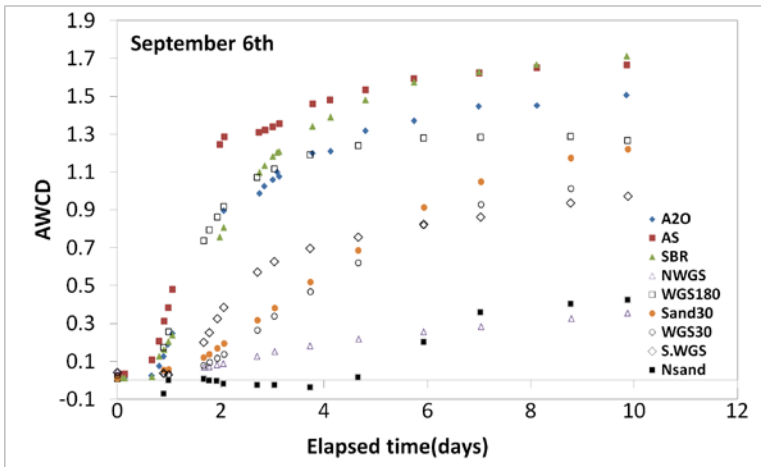


Figure 4.2 –Temporal changes of AWCD for various sampling events.

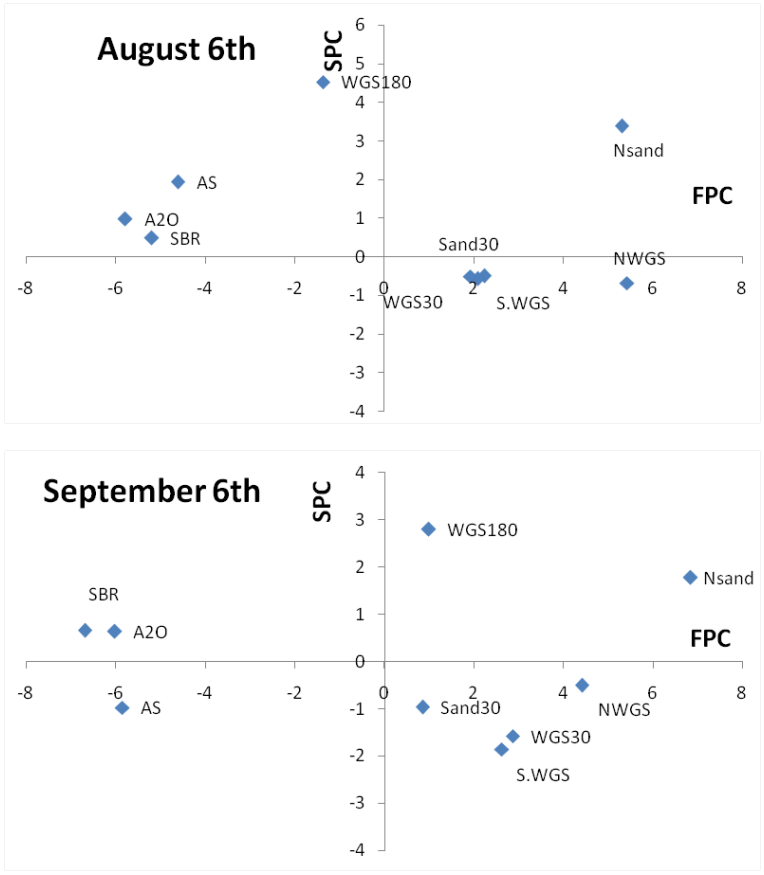


Figure 4.3 –Changes of microbial substrate metabolic patterns at each sampling event (August 6th and September 6th).

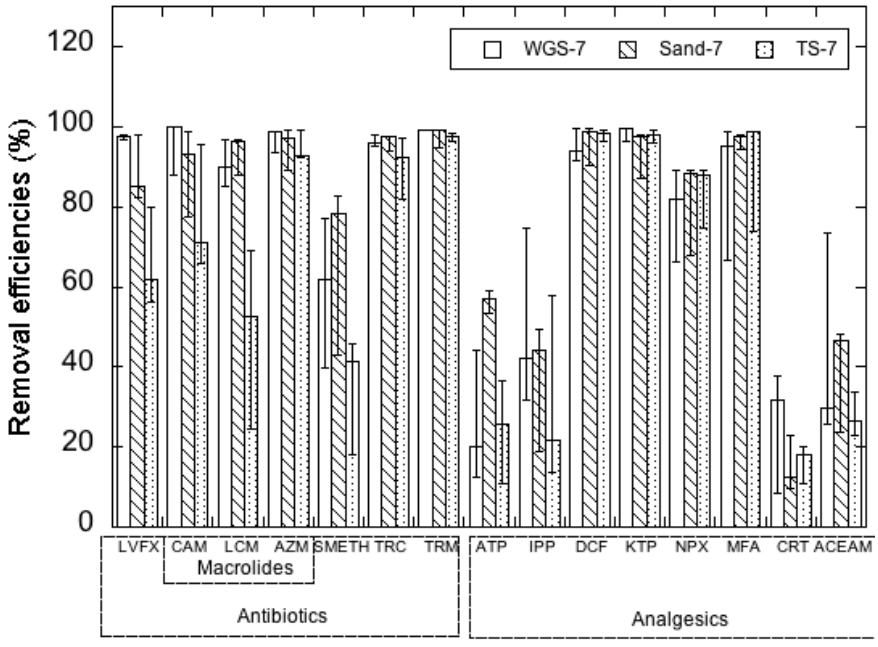
4.3.2. Effects of packing materials on the removals of PPCPs

In this section, the effects of packing materials on the sorption and biodegradation of PPCPs were evaluated. Removal efficiencies of selected PPCPs in WGS, sand and TS were shown in Figure 4.4. Among antibiotics, LVFX, one of fluoroquinolones, was removed by 97.7%, 85.3%, and 61.7% in WGS-7, Sand-7, and TS-7, respectively. Similarly, effective removals (above 90%) were observed for CAM, LCM, and AZM in WGS-7 and Sand-7. In TS-7, the removal efficiencies of CAM, LCM and AZM were 71.1%, 52.5% and 92.7%, respectively. In addition, TRC (K_{oc} = 2400, pK_a = 7.9) were removed effectively in WGS-7, Sand-7, and TS-7 (96.1%, 97.7%, and 92.2%, respectively).

Among the analgesics and antilipidemics PPCPs, the removal efficiencies of DCF, KTP, NPX, MFA, BZF, CFB, and GFB were in the range of 81.8% and 99.6% (in WGS-7); 80.6% and 99.0% (in Sand-7); 84.9% and 98.7% (in TS-7). Their removals in SAT are all efficient regardless different packing materials. In addition, the removal efficiencies of FSM, another carboxylic compound, were 94.4%, 98.8%, and 99.6% in WGS-7, Sand-7, and TS-7, respectively. The removal efficiencies of DEET were 70.3%, 55.3%, and 54.2% in WGS-7, Sand-7, and TS-7, respectively.

For amide PPCPs (ATP, IPP, CRT, PRM, and CBM), their removals were poor regardless packing materials (less than 50%) except the removal of ATP in Sand-7 (57.2%). Furthermore, the concentration of CBM increased in SAT columns with different packing materials (78.8%, 114.0% and 52.5% in WGS-7, Sand-7, and TS-7, respectively).

In summary, WGS was more effective on the removals of antibiotics, but no clear differences were found for the removals of biodegradable PPCPs and amide PPCPs in SAT among different packing materials.



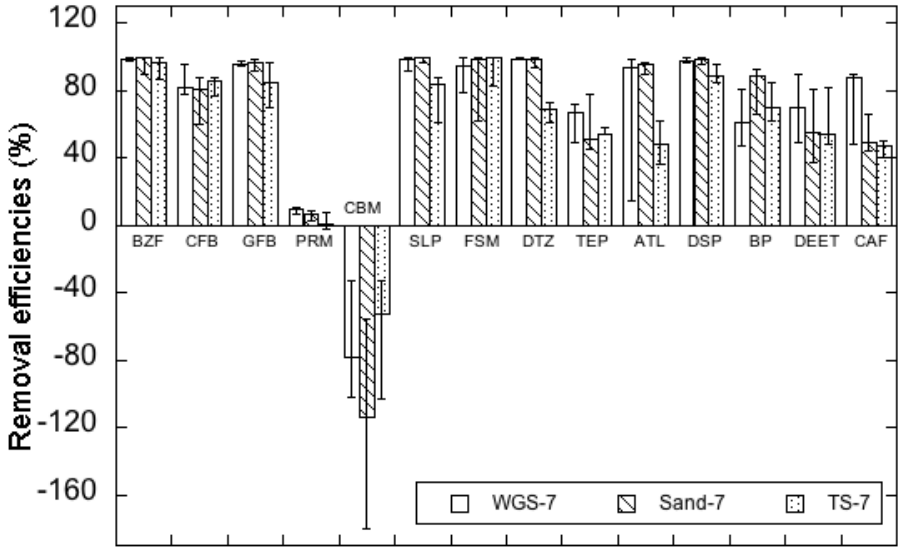


Figure 4.4 –Removal efficiencies of PPCPs in SAT columns with different packing materials (WGS, Sand, and TS).

4.3.3. The effect of HRT on PPCP removals in SAT

The removal efficiencies of target PPCPs in sand columns and WGS columns in the first part (relatively long HRT) were shown in **Figures 4.5** and **4.6**. The removals of LVFX were efficient in sand and WGS columns regardless HRT (above 83.5%). In addition, the removals of CAM, LCM, AZM, TRC, and TRM were highly efficient (above 90%) under the conditions above. Antibiotics expect SMETH were removed effectively in SAT with different HRTs. For SMETH, its removal efficiencies were improved by extending HRT in WGS columns (61.8%, 72.7%, and 81.5% in WGS-7, WGS-30, and WGS-180, respectively).

For easily biodegradable PPCPs (DCF, KTP, NPX, MFA, BZF, CFB, GFB, and FSM), their removals in WGS and sand columns with different HRT were all efficient (above 80%). More specifically, the removal of NPX was efficient in SAT with an HRT of 7 days or longer (81.8%, 83.8% and 87.5% in WGS-7, WGS-30, and WGS-180, respectively). For biodegradable PPCPs except NPX, highly effective removals were expected in WGS-7. For other PPCPs (SLP, DTZ, ATL, and DSP), their removals were similar among different HRTs. For other moderately biodegradable compounds, the removal of TEP was improved by extending HRT (from 67.3% to 82.4% in WGS); the removal efficiencies of BP ranged from 61.5% to 94.7% by extending HRT.

For amide PPCPs such as ATP, IPP, CRT, and PRM, their removals were poor in WGS-7 (20.0%, 42.2%, 31.9%, and 9.3%, respectively), and the clear improvements on their removals were not observed by extending HRT (28.7%, 40.4%, 49.2%, and 14.6% in WGS-180, respectively). These results indicate that extending HRT cannot improve the removals of amide PPCPs in SAT.

In summary, during long-term (i.e., HRT ≥ 7 days) operation, effective removals were observed for most PPCPs in WGS column with an HRT of 7 days, and extending HRT showed little influence on the removals of PPCPs.

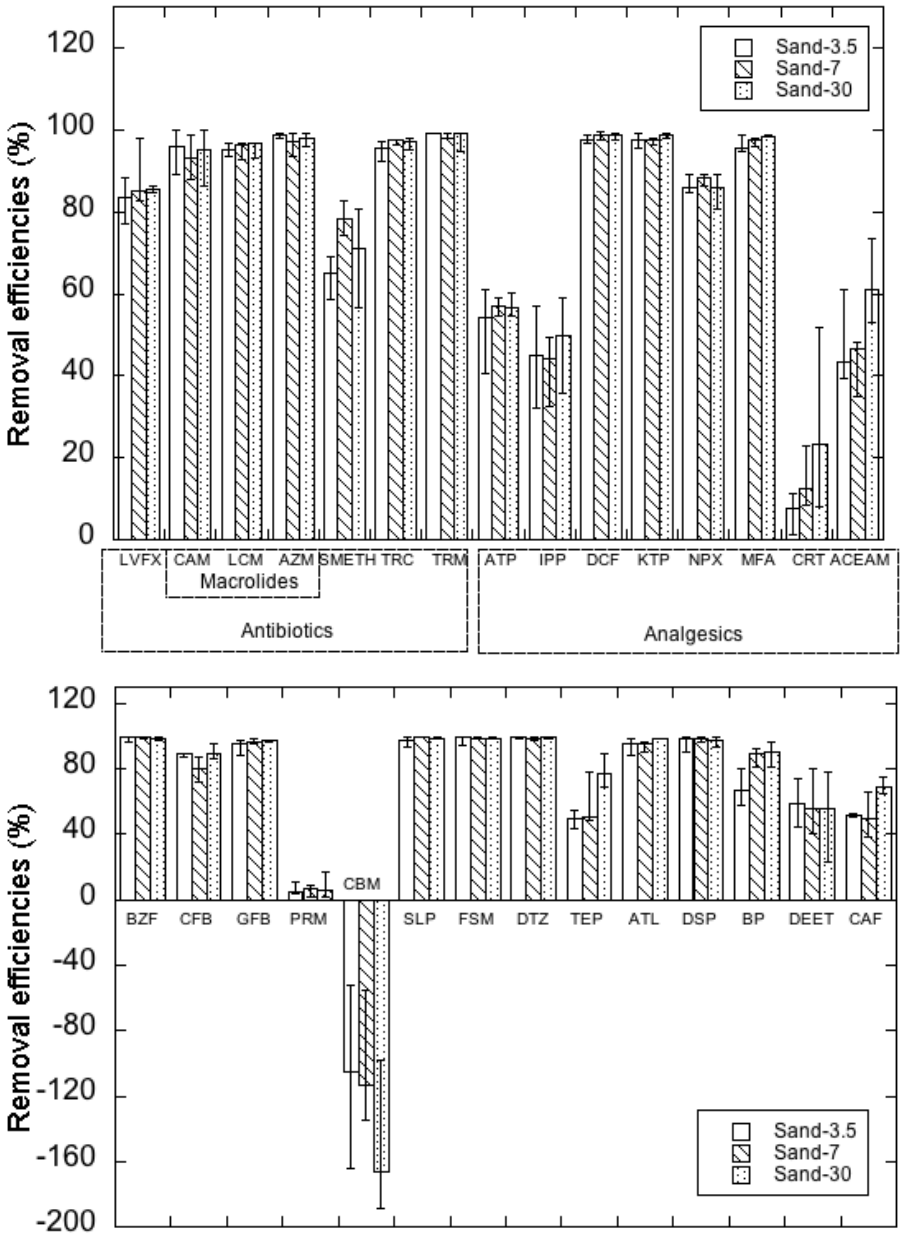


Figure 4.5 –Removal efficiencies of PPCPs in sand columns with different HRTs (3.5, 7, and 30 days).

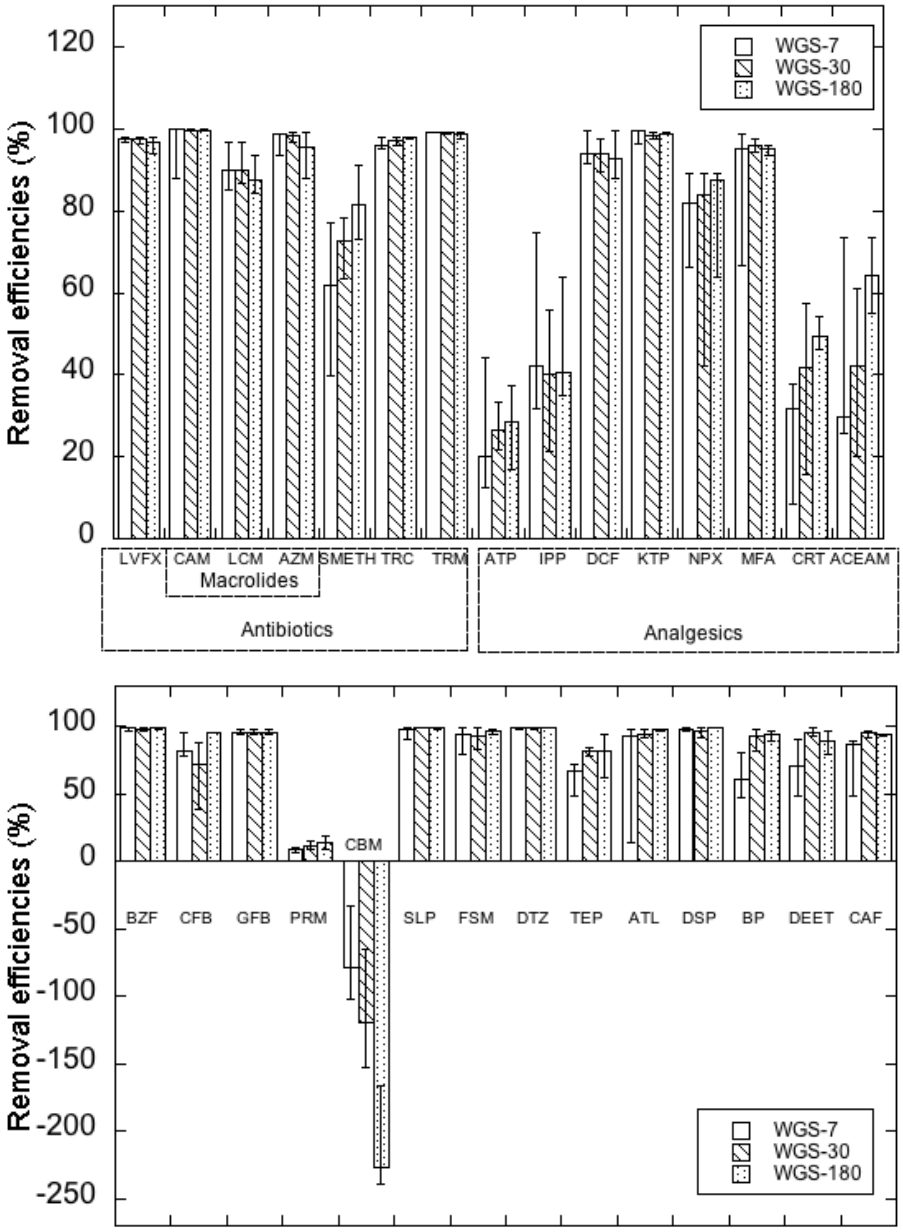


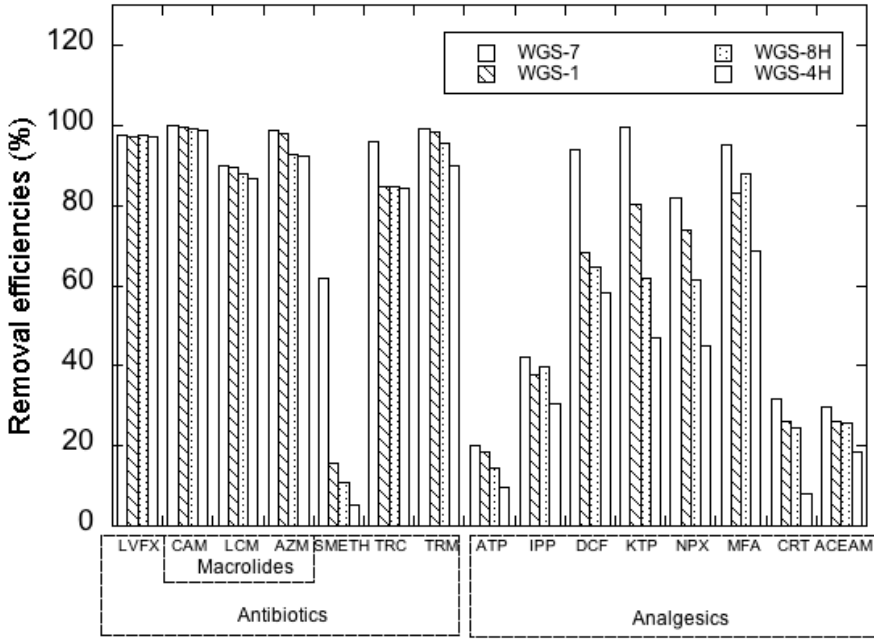
Figure 4.6 –Removal efficiencies of PPCPs in WGS columns with different HRTs (7, 30, and 180 days).

As presented above, a long HRT showed little influence on the sorption and biodegradation. In the second part, short-term column experiments were operated (Figure 4.7). For antibiotics, LVFX, CAM, LCM, AZM, TRC, and TRM were still removed effectively (above 90%) even in WGS column with a short HRT (WGS-1, WGS-8H, and WGS-4H, respectively), but the removals of SMETH were poor in columns with short HRT (15.6%, 10.9%, and 5.1% in columns of WGS-1, WGS-8H, and WGS-4H, respectively).

For biodegradable PPCPs (DCF, KTP, NPX, MFA, BZF, CFB, GFB, and FSM), the removal efficiencies increased by extending HRT. For example, removal efficiencies of KTP were 47.1%, 62.0%, 80.4%, and 99.6% in WGS-4H, WGS-8H, WGS-1, and WGS-7, respectively. These indicated that HRT had a positive influence on the removals of biodegradable PPCPs in short-term operation. For amide PPCPs, the removals of ATP, CRT, and ACEAM were improved by extending HRT, but extending HRT did not improve the removals of PRM, CBM and IPP in SAT.

In Figure 4.8, the removal efficiencies of different PPCP categories were summarized by calculating the total concentration of each category in the influent and effluent from each column. Antibiotics were removed effectively even in WGS columns with short HRTs. The removals of analgesics, antilipdemics and others were improved by extending HRT. For an HRT of 7 days, the removal efficiencies of all selected categories reached above 60%.

In short-term operation, high removals of antibiotics were observed, and extention of HRT showed positive influence on the removals of analgesics and antilipdemics. PRM, CBM and IPP were persistent in SAT even with a long HRT.



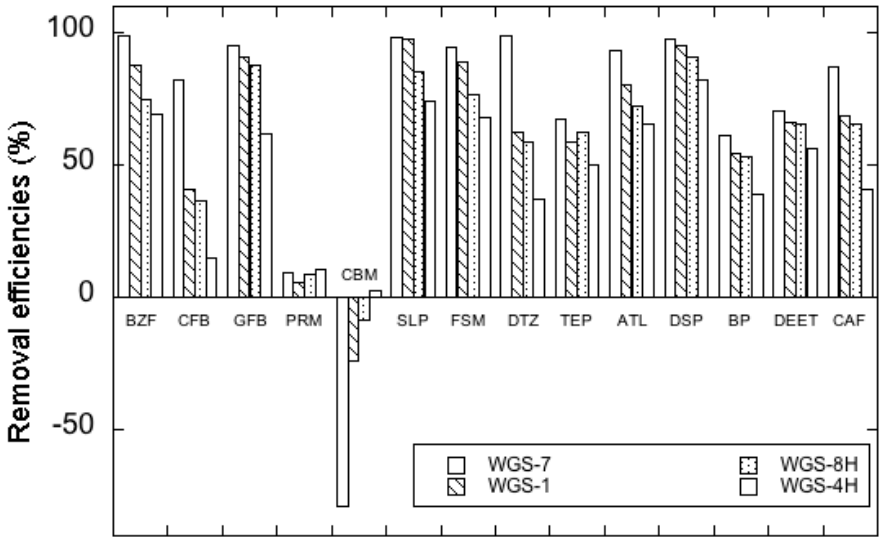


Figure 4.7 –Removal efficiencies of PPCPs in WGS columns with short HRT (4 hrs, 8 hrs, 1day and 7 days).

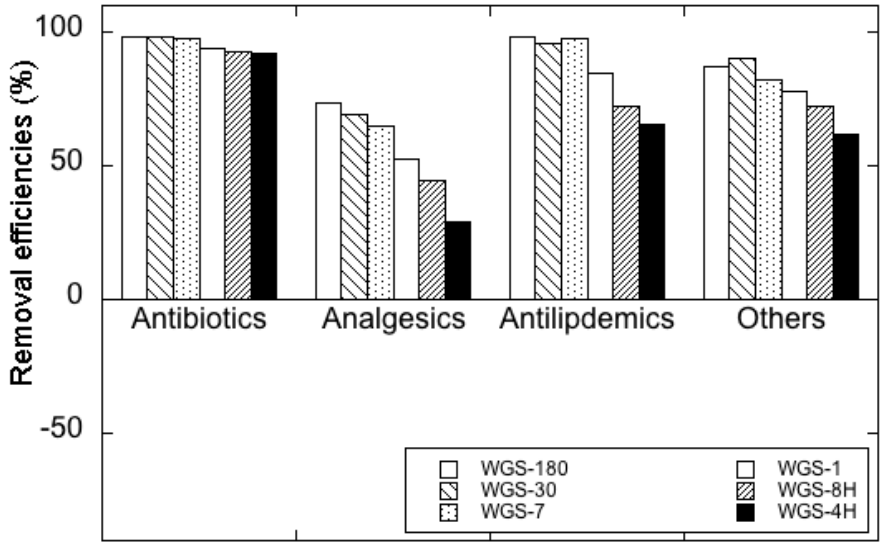


Figure 4.8 –Removal efficiencies of PPCPs with respect to classification in WGS columns with HRTs.

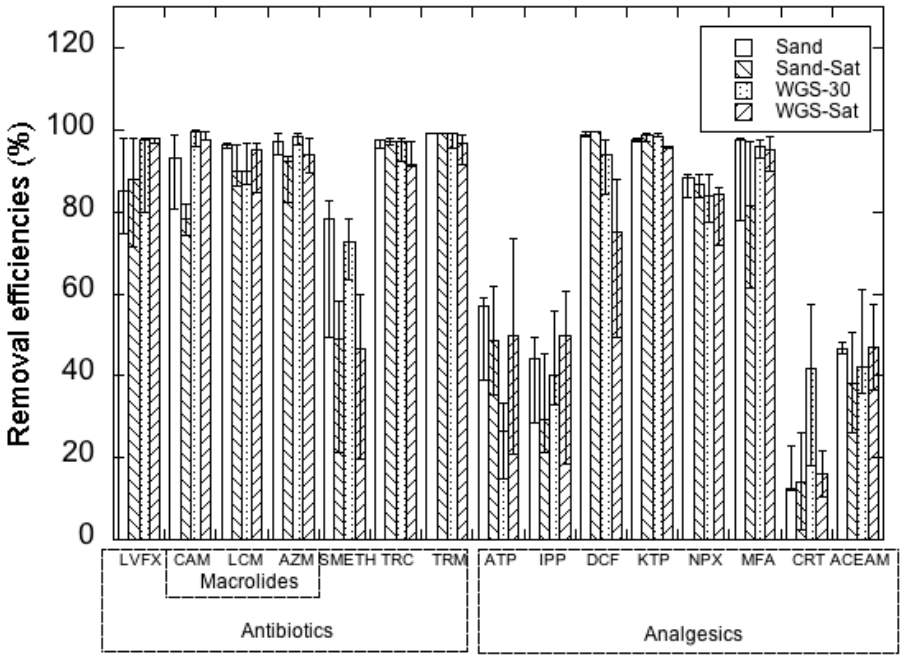
4.3.4. The effect of saturation condition on PPCPs removals in SAT

In this section, Sand and WGS columns under saturated and vadose conditions were operated. The removal efficiencies of target PPCPs in sand and WGS columns under saturated and vadose conditions were shown in Figure 4.9.

For LVFX, CAM, LCM, AZM, TRC and TRM, their removals were high (above 80%) under both vadose and saturated conditions. For easily biodegradable PPCPs (DCF, KTP, NPX, MFA, BZF, CFB, GFB, and FSM), their removal were high (above 80%) except DCF in WGS-Sat. For amide PPCPs (ATP, IPP, CRT, PRM, and CBM), their removals were consistently poor (less than 50%) both under saturated and vadose conditions.

Though most selected PPCPs behaved similarly on their removals under saturated and vadose conditions, the removal efficiencies of SMETH were 46.4% and 72.7% in WGS-30 and WGS-Sat, 49.0% and 78.4% in Sand-30 and Sand-Sat, respectively.

In summary, the removals of most PPCPs except SMETH were similar in SAT under saturated and vadose conditions.



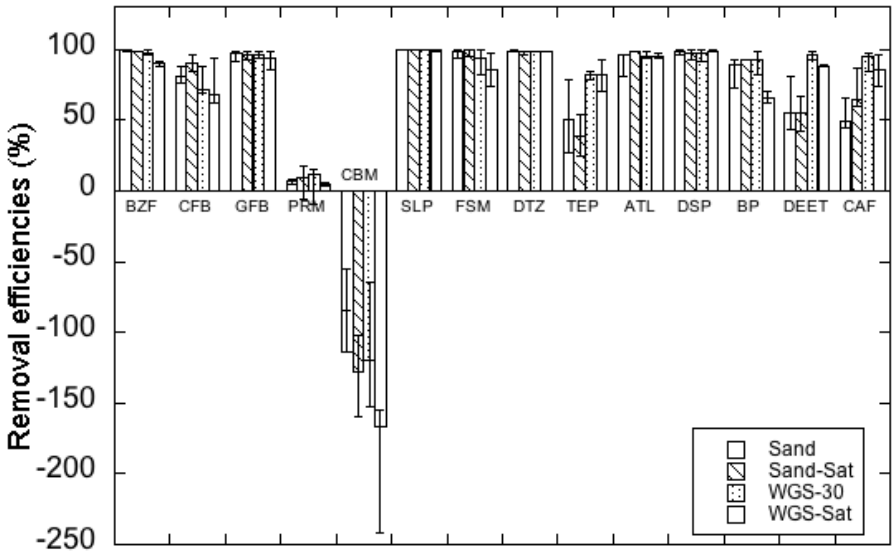


Figure 4.9 –Removal efficiencies of PPCPs in SAT columns under saturated and vadose (unsaturated) conditions.

4.4. Discussion

4.4.1. Microbial substrate metabolic patterns

In BIOLOG analysis, the difference of metabolism patterns was identified by the locations of samples in PCA analysis of BIOLOG [10, 11], and the metabolic pattern of SAT soil samples (WGS180, WGS30, Sand30 and S.WGS) was at a midpoint between the activated sludge samples (AS, SBR, and A_2O) to the non-used packing materials (NWGS and Nsand). These indicate that biological communities in SAT were metabolically different from activated sludge and natural soil. Pavelic et al.[12] demonstrated that bacteria accumulation occurred in SAT by feeding secondary effluent. Takabe et al.[7] observed that the top layer in SAT (0~5 cm from the soil surface) showed different characteristics of biodegradation compared with AS treatment, indicating that the biological communities in SAT were influenced by A_2O water, but different from AS treatment. Furthermore, the BIOLOG results in this study indicated that many organic compounds not removed effectively by activated sludge (i.e., amino acids (L-Arginine, L-Phenylalanine, L-Serine) and polymers (Tween 40)) could be removed by microorganism in the SAT. In addition, the comparison between S.WGS and WGS30 indicated that the metabolite patterns in vadose zone and saturated zone were relatively similar, which was also in agreement with the removal characteristics of PPCPs under these two conditions. However, metabolic pattern demonstrated by WGS180 was unique compared with other SAT samples. As discussed in Part 4.4.2, a long HRT was helpful for the biodegradation of ACEAM, TEP, and BP, which were not biodegraded in SAT with a short HRT. For the biological communities in SAT column with a long HRT, Idelovitch et al. [13] mentioned that biodegradation of organic substances required a long period of time for the development and acclimatization of the microbial population

capable of degrading them. The growth of the adaptable biological communities could be expected in SAT by extending HRT [14, 15]. These indicated that extention of HRT could contribute to the growth of different biological communities in SAT.

As shown in Table 4.4, activated sludge samples (A_2O, AS, and SBR) were similar in the metabolism of substrates. More specifically, carbohydrates and carboxylic acid were biodegradable in activated sludge samples. From the perspective of functional groups, most biodegradable PPCPs (i.e., KTP, BZF, and CFB) in SBR have carboxyl groups. These indicated that carboxyl groups contributed to the biodegradabilities of compounds. The microbial substrate metabolic patterns in SAT samples covered a wider range of compounds than those in activated sludge samples: amino acid, amines, and polymers were biodegradable in SAT besides carbohydrates and carboxylic acid. In SAT, the biodegradation of SMETH (amine) and DCF (amine) were expected. Furthermore, amino acids were also biodegradable in SAT and soil treatment [16, 17]. These trends were in agreement with the removal trend of PPCPs in SAT. Thus, SAT possess a wider range of biodegradation, although the biodegradation rate was lower than activated sludge samples.

4.4.2. The influence of packing materials on the removals of PPCPs

As discussed in Chapter 3, sorption was expected in WGS columns for the removals of fluoroquinolones. Picó et al. [18], Golet et al. [19], and Uslu et al.[20] reported that poor biodegradation and resistance of fluoroquinolones were expected in solid matrices. Thus, their high removals in sand and TS were attributed to the sorption in these packing materials. Among selected packing materials, WGS performed better for antibiotics removals than sand and TS, and this could be attributed to the following factors: Firstly, WGS have a higher TOC value (0.0872%, shown in Table 3.1) than sand and TS. Steven et al. [21] demonstrated that the difference of packing materials on the removals of antibiotics could be attributed to hydrophobic interaction between soil organic matter (SOM) and antibiotics. Yu et al. [22] mentioned that organic content and particle size distribution influenced the sorption of micropollutants in soil. However, TS have higher TOC values (0.0144%) than sand (0.0094%). For the characteristic related to sorption, not only organic content of soil influenced the sorption of micropollutants in soil [22], but also surface characters of soil such as specific surface area strongly enhanced the sorption [23, 24]. In this study, WGS and SAND have much higher values of specific area (5.95 and 2.40 m^2/g) than TS (0.812 m^2/g). This can explain the difference of removal efficiencies of LVFX between Sand-7 and TS-7. Secondly, WGS had a higher CEC value (15.6 meq/100mg, seen in Table 3.1). CEC is the index of positive charges (cations). Usually, cation exchange for antibiotics is expected in soil [25]. Gao et al. [26] found that CEC had a positive influence on the sorption of sulfonamide antimicrobial agent to clay minerals. Sassman et al.[27] mentioned that cation exchange would dominate the sorption process in soil. In addition, WGS, sand and TS were similar with respect to pH, porosity, and particle size distribution. Thus, high removals of antibiotics in WGS were attributed to high TOC and CEC.

As discussed in Chapter 3, biodegradation is another main mechanism of the removals of PPCPs. In this chapter, biodegradable PPCPs were removed efficiently

regardless soil types (WGS, sand and TS). For poorly biodegradable PPCPs, their poor removals did not vary with different packing materials. Kasprzyk-Hordern et al. [28] also demonstrated that CRT and CBM were removed only slightly in soil infiltration. These indicated that the characteristics of biodegradation were similar regardless packing materials. Biological communities in SAT were strongly influenced by the biological communities in A_2O water [7], so the biological communities in different packing materials columns with feeding the same A_2O water could be similar.

In summary, sorption process in SAT was influenced by soil properties (i.e., K_{oc} and pK_a), but biodegradation in SAT does not rely on the properties of the packing material.

4.4.3. The influence of HRT on the removals of PPCPs

The extention of HRT improved the removals of micropollutants in SAT as presented in Chapter 2, but the extention of HRT requires unrealistically a large space for treatment. Thus, the influence of HRT would be discussed to find a suitable HRT for controlling micropollutants. For the removals of PPCPs in SAT, the removal efficiencies of antibiotics except SMETH were high regardless HRTs. Rapid sorption of compounds having high K_{oc} value (e.g., antibiotics) was expected [29, 30]. Thus, the sorption process is fast in SAT, and HRT showed little influence on their sorption process in SAT.

Carboxylic PPCPs in analgesics and antilipdemics (DCF, KTP, NPX, MFA, BZF, and GFB) were easily biodegradable in SAT as discussed in Chapter 3. Except NPX, their removal efficiencies were high enough in WGS-7. Abel [31] mentioned that SAT with several days (5 days) can remove pharmaceutically active compounds (PhACs) effectively. Ternes et al.[32] reported that IBP and BZF were readily removed at above 90% in SAT with an HRT of less than 1 day. However, the fact that removals of these carboxylic PPCPs in short-term operation increased with longer HRTs (from 4 hrs to 7 days) indicated that several hrs were not sufficient for the control of carboxylic PPCPs. For biodegradation, Drewes et al. [33] also reported that treatment facilities employ a shorter HRT during secondary treatment tend to show lower removals of DOC. Matamoros et al. [34] mentioned that extention of HRT led to an increase in PPCP concentration in the outlet. Zhang et al. [35] demonstrated that biodegradations of estrogen in soil columns were enhanced by extending HRT. These indicated that effective biodegradation of micropollutants was formed by the sufficient HRT in SAT.

As discussed in Chapter 3, SMETH was moderately biodegradable, and its removal was moderately effective in WGS-7 but effective in WGS-180. Gimbel et al. [36] mentioned that extention of HRT was helpful for the removal of SMETH. Stoob et al. [37] reported that the concentration of spiked SMETH decreased with increasing contact time in soil. SMETH possesses sulfonamide group, and the persistence of sulfonamide compounds was observed in soil [38-40]. Thus, the effective removal of SMETH in WGS columns was attributed to a sufficient HRT.

ACEAM is biodegradable according to previous studies on SAT [14, 41]. However, in this study, the concentration of ACEAM in A_2O water was 34 ng/L, which was lower than that in the influent of WWTPs (108 ng/L on average). Thus, a long HRT

in SAT contributed to its further removal even with low concentrations. For TEP and BP, extending HRT was also helpful for the improvement on their removals. Drewes et al.[33] mentioned that a longer HRT in SAT eliminated organic pollutants effectively. In addition, a long HRT (180 days) was helpful for improving the removal of CRT, which was resistant in WGS-7. However, for ATP, IPP, PRM and CBM, extending HRT showed little influence on their removals in SAT because of their poor biodegradabilities. Overall, by extending HRT in SAT, the removals of moderately biodegradable compounds could be more easily improved than the removals of amide PPCPs.

Though SAT with several months, even several years can remove PPCPs at high removal efficiencies, for example, above 99% [42-44], the long HRT limited the treatment capacities of SAT. Thus, the balance between the removals of micropollutants and treatment space should be evaluated in the operation of SAT. The environmental risk of its effluent will be discussed in Part 4.4.5.

In summary, a short HRT (7 days) was sufficient for the sorption of antibiotics expect SMETH. The effective removal of SMETH was attributed to the long SAT in SAT. The removals of amide PPCPs (ATP, IPP, PRM, and CBM) were poor regardless HRT, and the removals of moderately biodegradable PPCPs (ACEAM, TEP, and BP) were improved by extending HRT. AN HRT of several hrs was not suitable for the effective removals of PPCPs, and WGS-7 can remove most of target PPCPs effectively. However, HRTs longer than 7 days does not improve the removals of most PPCPs too much.

4.4.4. The influence of saturated condition on the removals of PPCPs

As presented in Chapter 2, vadose condition was another important operating condition besides the packing materials and HRT. According to the results, for the antibiotics removed by sorption and ionic interaction, their removal efficiencies did not vary under vadose or saturated conditions.

For carboxylic compounds, their removal efficiencies were efficient or highly efficient under both saturated and vadose conditions. Conkle et al.[45] mentioned that the half-lives of GFB and IBP in wetland under anaerobic condition was longer than under aerobic condition, and the existence of oxygen is helpful for their removals[45]. Jim et al.[46] reported that 13 of 18 selected PPCPs including ACEAM, DCF, IBP, GFB, NPX, and KTP, were effectively biodegraded under aerobic conditions. Lin et al.[47] reported that NPX, TRM, DCF, IBP, and SMETH were susceptible to biodegradation under aerobic condition. These indicated that aerobic condition is helpful for the biodegradation of most biodegradable PPCPs. However, in this study, SAT under saturated condition showed similar removal efficiencies of profens and antilipidemics as SAT under vadose condition. In SAT, easily biodegradable PPCPs such as profens and antilipdemics was removed more effectively in short contact time than other PPCPs. The concentration of DO in the feed water was 3.36 mg/L on average [7], so relative aerobic condition was maintained in the top level of saturated column. Thus, the biodegradation of easily biodegradable PPCPs could be effectively removed in the top of saturated column. For poorly biodegradable PPCPs, such as PRM and CBM, their removals were similarly poor under saturated and vadose conditions. Park et al. [48] observed that CBM was resistant

(<5%) in wetlands under anoxic condition. Li et al. [49] reported that the degradation of CBM did not exceed 2% in soil through 120 days of incubation under aerobic conditions. Conkle et al. [45] demonstrated that CBM was resistant in wetland sediments under aerobic and anaerobic conditions. Drewes et al. [50] reported that CBM and PRM were not removed during ground water recharge under either anoxic saturated or aerobic unsaturated condition. Lin et al. [51] also mentioned that PRM was persistent in soil under aerobic and anaerobic condition. Saturated condition and vadose condition have little influence on the biodegradation of poorly biodegradable PPCPs.

Though most selected PPCPs were similar on their removals under saturated and vadose conditions, SMETH and CRT were removed more efficiently under the vadose condition in SAT. The vadose condition was helpful to make the air trapped in soil and increase the oxygen concentration in the soil to support the aerobic biodegradation of SMETH. Monteiro et al.[2] mentioned that aerobic bacteria contributed to the removals of SMETH in soil. Liu et al.[52] and Baumgarten et al. [53] also reported that aerobic bacteria can consume oxygen easily under vadose condition to degrade SMETH. Different from profens and antilipdemics, the removal of SMETH reached the effective level in WGS-180 column, and this indicated that the biodegradation of SMETH need long-term contact time as discussed in Part 4.4.3. Relatively high DO concentration was maintained in the deep levels in SAT under vadose condition [7], so aerobic condition was expected in the whole SAT columns to support the biodegradation of SMETH and CRT.

In summary, easily biodegradable PPCPs such as profens and antilipdemics were removed effectively regardless of vadose conditions, but the removals of SMETH and CRT were improved under vadose condition. For practical use with the same area, the capacity of SAT under saturated condition is larger than that under vadose condition. Thus, saturated condition could be a practical choice for controlling micropollutants in SAT and A_2O water after aerobic treatment could support the biodegradation in SAT.

4.4.5. Environmental risk of PPCPs in SAT

As present above, extending HRT improved the removals of moderately biodegradable PPCPs such as halogenated acid in SAT, but high removals of most selected PPCPs in SAT were achieved with an HRT of 7 days. From a practical point of view, extention of HRT required huge treatment space for SAT. The balance of water quality and practical benefit should be considered referring to the risk evaluation. In this study, SAT was used for indirect potable use in wastewater reclamation, and the environmental risk was evaluated. In Chapter 2, environmental risks of PPCPs in aquifer treatment were presented. In this section, WGS-7 were selected as target SAT column, and the environmental risk of its effluent was evaluated referring to the PNEC analysis [54]. According to the concentrations of PPCPs in A_2O water, 29 PPCPs in Table 2.2 were selected to evaluate the change of environmental risk of PPCPs before and after SAT.

In Figure 4.10, the MEC-to-PNEC ratios for CAM, LCM, AZM, TRC, KTP and DEET in A_2O water were relatively high (i.e., 24.93, 0.44, 6.23, 6.42, 6.15, and 0.11,

respectively). The MEC-to-PNEC ratios for CAM, AZM, TRC, and KTP in A_2O water were above 1. After SAT in the WGS-7 column, the MEC-to-PNEC ratios of most PPCPs were below 0.1, and the only PPCP with a MEC/PNEC value above was TRC (0.25).

Referring to the work of García et al. [55], the levels of toxicity were divided into 3 levels with respect to the ratios (R) of MEC/PNEC as follows: (i) High toxicity: R>1; (ii) medium toxicity: 0.1<R<1, and (iii) low toxicity: R<0.1. The MEC-to-PNEC ratios for LCM and DEET indicated their occurrence in A_2O water belonged to the group of medium toxicity. Furthermore, the occurrence of CAM, AZM, TRC, and KTP in A_2O water belonged to the group of high toxicity. These indicated that environmental risk of PPCPs should be considered in wastewater effluent. After treatment of WGS-7, the ratios of MEC/PNEC of all selected PPCPs except TRC (0.25) were below 0.1. However, the ratio of MEC/PNEC of TRC was 0.13 in WGS-180. This indicated that WGS-7 could be sufficient to control the environmental risk of PPCPs in wastewater effluent.

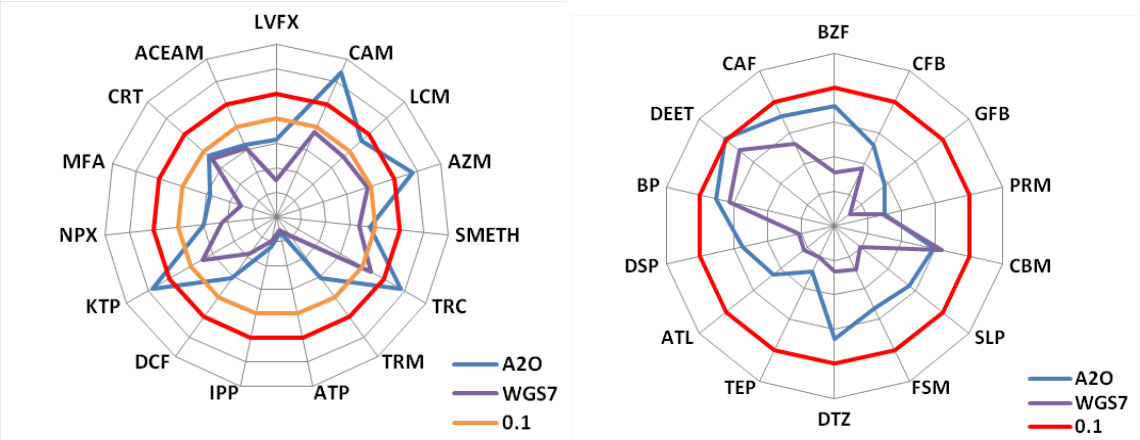


Figure 4.10 –Preliminary estimation of the risk (MEC/PNEC) of PPCPs in A_2O water and SAT effluent (WGS-7).

4.5. Summary

For biodegradation pattern, SAT showed higher biological activities than natural unused soil because of feeding A_2O water. SAT has a wider range of biodegradation than AS treatment with the extention of HRT.

WGS possessing high values of TOC, CEC and specific area was available to remove antibiotics by sorption, but packing materials had little influence on the biodegradation characters in SAT.

For the effect of HRT in SAT, in WGS columns with a short-term HRT, removal efficiencies of biodegradable PPCPs (i.e., carboxylic PPCPs) was improved by extending HRT, but extention of HRT from 7 days only improved the removals of SMETH, ATP, CRT and ACEAM in SAT. For most PPCPs, their removals were sufficiently high in the WGS column with an HRT of 7 days.

For vadose condition, the removals of most selected PPCPs were similar under vadose and saturated conditions, but the removals of SMETH and CRT were more effective under vadose condition than saturated condition. Aerobic condition was expected in the top levels of saturated column because of DO in wastewater effluent. From the perspective of practical use, cooperation of SAT and AS treatment (A_2O process) was suitable for wastewater reclamation to control micropollutants.

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Chapter 5 Pathways of transformation products in SAT and AS treatment

5.1. Introduction

Biodegradation played an important role in the removals of micropollutants in SAT and AS treatment. However, it is common that these treatments have inability to mineralize biodegradable micropollutants completely, and transformation products (TPs) were expected after biodegradation. In this chapter, the formation of TPs of CAF and 2-arylpropionic acids was evaluated as indicators for addressing the pathway of biodegradation in SAT and AS treatment. Furthermore, from the perspective of the chemical structures, many micropollutants possess methyl and carboxyl groups in common as described in Table 2.1, and the functional groups influence the transformation of micropollutants [1]. Among selected PPCPs, methyl and carboxyl groups widely existed in biodegradable PPCPs (Table 2.2), and PPCPs were the indicators of micropollutants in SAT and AS treatment as discussed in Chapter 3. Thus, the transformation pathways and transformation processes of PPCPs with methyl and carboxyl groups in SBR and SAT columns are compared to understand the difference of biodegradation in AS treatment and SAT with respect to the pathway of biodegradation.

Among selected PPCPs in this study, CAF possesses three methyl functional groups in its chemical structure, and it was biodegradable in AS treatment and SAT. In biological metabolism, consecutive demethylation was considered as the main transformation pathway of CAF [2]. Thus, CAF is an ideal indicator to evaluate the demethylation of micropollutants with methyl function groups. In addition, 2-arylpropionic acids (profens) are widely used carboxylic acid-containing drugs possessing arylpropionic acid as a common structure. Thus, profens are ideal indicators to evaluate the transformation of micropollutants with carboxyl groups.

In this chapter, firstly, the demethylation pathways of CAF in SAT and AS treatment were compared, followed by the concentrations of CAF TPs in A_2O water and SAT effluent. Secondly, transformation processes of profens between SAT and AS treatment were evaluated as well as the chemical structures of their TPs and the occurrence of TPs in these two treatments. In summary, the cooperation of AS treatment and SAT would be understood on basis of the difference of biodegradation pathway.

5.2. Materials and methods

In this chapter, biodegradation the pathway of CAF and the biodegradation processes of profens were investigated. For CAF TPs, the analysis was performed with their standard compounds. SBR and batch experiments with SAT biomass suspension were used for the comparison of CAF demethylation pathways, and the occurrences of CAF TPs in lab-scale WGS columns were evaluated. For profen TPs, their analysis was performed without standard compounds. The formation characteristics of profen TPs in SAT and AS treatment were compared by adding each profen in soil batch-treatment experiment and SBR.

5.2.1. Reagents

In addition to the reagents mentioned in Chapters 3 and 4, paraxanthine, theobromine, theophylline, 1-methylxanthine, 3-methylxanthine, 7-methylxanthine, and xanthine (hereinafter called as PX, THB, TEP, 1-MX, 3-MX, 7-MX, and XAN, respectively) were chosen as target TPs of CAF demethylation. FNP, NPX, KTP, and IBP were chosen as target profens to evaluate the transformation pathway of profens because of their common part of arylpropionic acid and their biodegradability in AS treatment and SAT.

3-MX, THB, XAN, and 3-benzoylphenylacetic acid (demethylated KTP) were purchased from Wako Pure Chemical Industries, Ltd.. 1-MX was purchased from Tokyo Chemical Industry, Co.. 7-MX was purchased from Sigma-Aldrich Co.. PX was purchased from Toronto Research Chemicals, Inc.. Dihydro KTP was purchased from Santa Cruz Biotechnology, Inc..

5.2.2. Experimental procedure

5.2.2.1. Columns setting

The columns used in this chapter have been mentioned in 4.2.2.1.

5.2.2.2. Biodegradation experiment for TP formation

(a) The formation of CAF TPs

In order to evaluate the difference of TPs formation between SAT and AS systems, biodegradation experiments of CAF were operated in reference to the work of Quintana et al. [3] and the OECD guideline [4] with minor modifications as follows.

For simulating biodegradation in SAT, the biodegradation experiments were operated in 4 L glass bottles containing 3 L of MQW, and CAF (final concentration = 1 μg/L) was spiked into the glass bottles. For eliminating of the influence of sorption on CAF, biomass suspension was prepared referring to the method described in 3.2. 80 mL of biomass suspension was added into the glass bottles. The runtime of biodegradation test was 5 days, and sampling times were 0, 1, 2, 3, 4, and 5 days. Samples were collected

at each sampling time and immediately filtered through 0.45 μm membrane filters (GF/75, ADVANTEC). The filtrates were stored at 4 ^{o}C until analyzed by LC-MS/MS.

For simulating biodegradation in AS treatment with the exclusion of the influence of sorption, activated sludge in the SBR was centrifuged for 1 min at 1000 rpm in a tabletop centrifuge (KUBOTA 5200, Kubota, Japan) to settle the sludge, and 1 μg/L of CAF was spiked into 100 mL of the supernatant. The runtime of SBR was 10 hrs in this experiment. Sampling times were 0, 2, 4, 6, 8, and 10 h. Samples were taken at each sampling time and immediately filtered through 0.45 μm membrane filters (GF/75, ADVANTEC). The filtrates were stored at 4 ^{o}C until being analyzed by LC-MS/MS.

(b) The degradation of CAF TPs

By spiking 1 μg/L of each CAF TP into SAT biomass suspension and SBR solution, the degradation rate of each CAF TP was evaluated referring to consecutive demethylation of CAF. Sampling times were 0, 1, 3, 5, and 7 days in SAT, and 0, 2, 4, 6, 8, 12 h in SBR, respectively, and samples were immediately filtered through 0.45 μm membrane filters (GF/75, ADVANTEC). The filtrates were stored at 4 ^{o}C until being analyzed by LC-MS/MS.

(c) TPs formation of Profens

SBR were applied for the study of profen TPs in AS treatment with the addition of 100 μg/L of each profen. Sampling times were 0, 1, 2.5, 4, 6 h.

For simulating the formation of profen TPs in SAT, the biodegradation experiments were conducted in 50 mL centrifuge tubes (Labcon, Petaluma, U.S.A) containing 30 mL of A_2O water and 10 g of wet soil from the WGS-30 reactor. Each profen (100 μg/L) was spiked into tubes, and tubes were shaked with a recipro shaker (SR-1N, TAITEC, Japan). The runtime of biodegradation experiments of profens was 9 days, and sampling times were 0, 1, 3, 7, and 9 days, respectively. Samples were also immediately filtered through 0.45 μm membrane filters (GF/75, ADVANTEC). The filtrates were stored at 4 ^{o}C until analyzed by LC-MS/MS.

5.2.3. Analytical methods

5.2.3.1. CAF TPs

Samples for CAF TPs were pretreated by SPE with the same method as presented in 3.2.7.1, and measured by LC-MS/MS. The conditions for CAF TPs in LC-MS/MS were shown in Table 5.1-Table 5.3.

Table 5.1 –LC-MS/MS conditions for CAF and its TPs.

LC system	Prominence 20A (Shimadzu)
Column	VP-ODS 4.6 μm 150×4.6mm(Shimadzu)
Column temperature	40 ^{o}C

LC condition	Sample cool temperature	4 °C
	Inject volume	20 µL
	Mobile phase	MQW/methanol
	Flow rate	0.8 mL/min
	Measurement time	26.5 mins
MS/MS condition	MS/MS	4000 QTRAP(AB SCIEX)
	Ionization mode	ESI+
	Curtain gas	40 psi
	Ionspray voltage	5500V
	Turbo ionspray temperature	700°C
	Nebulizer gas pressure	60 psi
	Turbo gas pressure	80 psi
	Collision gas	8

Table 5.2 –MRM conditions for CAF and its TPs.

	Q1/Q3	DP (V)	EP (V)	CE (V)	CXP (V)
CAF	195.036/138.000	70	10	34	11
TEP	181.082/124.000	60	10	32	19
THB	181.095/138.100	61	10	27	10
PX	181.098/124.000	61	10	29	8
1-MX	167.034/110.000	61	10	27	8
3-MX	167.052/124.000	61	10	25	8
7-MX	167.022/124.000	76	10	27	10
XAN	153.075/109.9	51	10	27	6

Table 5.3 –LC gradient condition for CAF and its TPs.

Time	Mobile phase (%)	
	MQW	Methanol
0	92	8
12	92	8
13	2	98
25	2	98
25.5	92	8

26.5	92	8

5.2.3.2. TPs of profens

To confirm the existence of TPs, the unique peaks in the chromatograms of spiked samples were considered as the peaks of TPs, and the fragment information of TPs was evaluated by using the mode of enhanced product ion (EPI) to identify their structures.

The script of Make MetID Methods.dll in Analyst Version 1.5.1 Software was used to establish the MRM method for potential TPs of each profen. Transformation list of Make MetID Methods.dll is shown in Table 5.4. LC gradient condition for profen TPs was the same as Table 3.8. The MRM method only provides the information of Q1/Q3 pairs. In the results of IBP transformation, oxidized TPs of IBP showed several peaks in the chromatogram. In order to identify the structure of these TPs, EPI scan (Q1 of 221.1) was used with the mass range of 50 and 220.

In addition, according to the results of KTP TPs by demethylation and hydrogenation, demethylated KTP and dihydro KTP were expected as the primary TPs of KTP, and the MRM conditions of demethylated KTP and dihydro KTP were shown in Table 5.5.

Table 5.4 –Transformation list in Make MetID Methods.dll.

Gain/Loss	Formula	Mass offset	Reaction name
1	O	15.995	Oxidation
2	O	31.990	Di-Oxidation
3	O	47.985	Tri-Oxidation
1	H_2O	18.011	Gain Of H_2O
1	H_2	2.016	Hydrogenation
-1	CH_2	-14.016	Demethylation
-1	H_2	-2.016	Dehydrogenation
1	SO_3	79.957	Sulfonation
1	CH_2	14.016	Methylation
2	CH_2	28.032	Di-Methylation
1	$C_6O_6H_8$	176.034	Glucuronidation
2	$C_6O_6H_8$	352.068	Bis-Glucuronide
1	$C_6O_6H_8SO_3$	255.991	Gluc-Sulphate
1	$C_6O_6H_8O$	192.029	Gluc-Oxidation
1	$C_{10}H_{15}N_3O_6S$	305.071	GSH
1	$C_6H_{10}O_5$	162.055	Glucose

Table 5.5 –MRM conditions for KTP and its TPs.

	Q1/Q3	DP (V)	EP (V)	CE (V)	CXP (V)
KTP	195.036/138.000	70	10	34	11
Demethyl KTP	181.082/124.000	60	10	32	19
Dihydro KTP	181.095/138.100	61	10	27	10

5.2.3.3. Recoveries and limitations of quantitation

Because CAF TPs have the same molecular formula and they have similar Q1/Q3 pairs in the MRM method, different TPs were separated by chromatography. In Figure 5.1, 1-MX, 3-MX, and 7-MX (methylxanthines) were separated clearly in this gradient method as well as THB, PX, and TEP (dimethylxanthines).

Five points above the LOQ were used for establishing the calibration curve, and a weight function of 1/*x* was selected for regression analysis. The average recoveries of target TPs were shown in Figure 5.2. Recoveries of CAF TPs ranged from 17.8% to 79.5% in A_2O water, and ranged from 20.9% to 83.3% in SAT effluent. The recoveries of 1-MX, 3-MX, and XAN were lower than other TPs (less than 30%), but were acceptable for their measurement [5, 6]. Their recoveries were not different between A_2O water and SAT effluent.

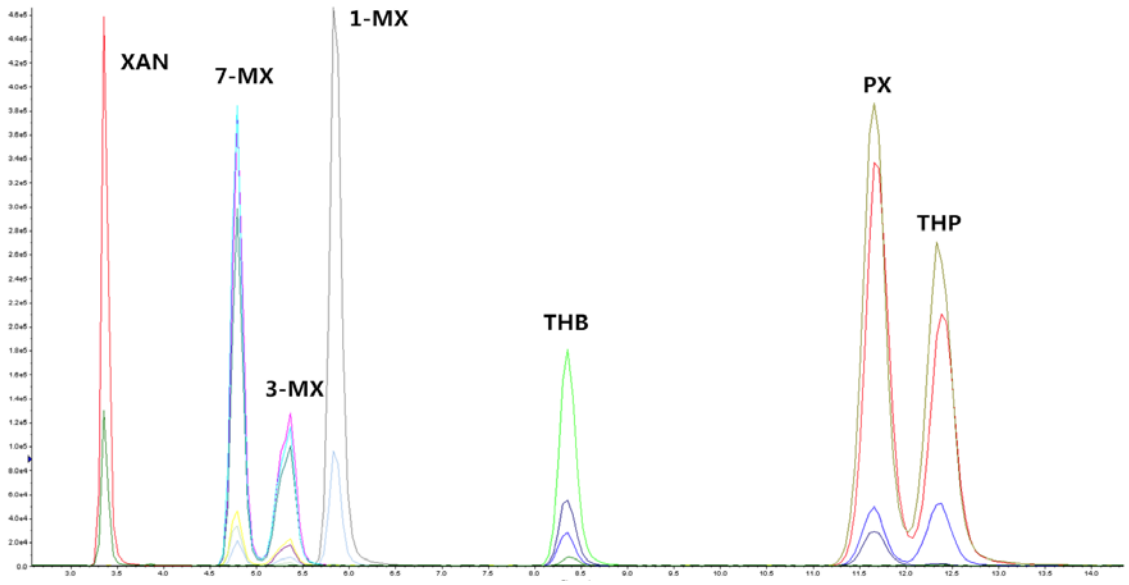


Figure 5.1 –The MRM chromatogram of CAF and its TPs.

Table 5.6 –LOQ of CAF and its TPs.

Compounds	LOQ (μg/L)	Compounds	LOQ (μg/L)	Compounds	LOQ (μg/L)
CAF	0.14	TEP	0.14	THB	0.08
PX	0.06	1-MX	2.08	3-MX	2.81
7-MX	0.69	XAN	2.43		

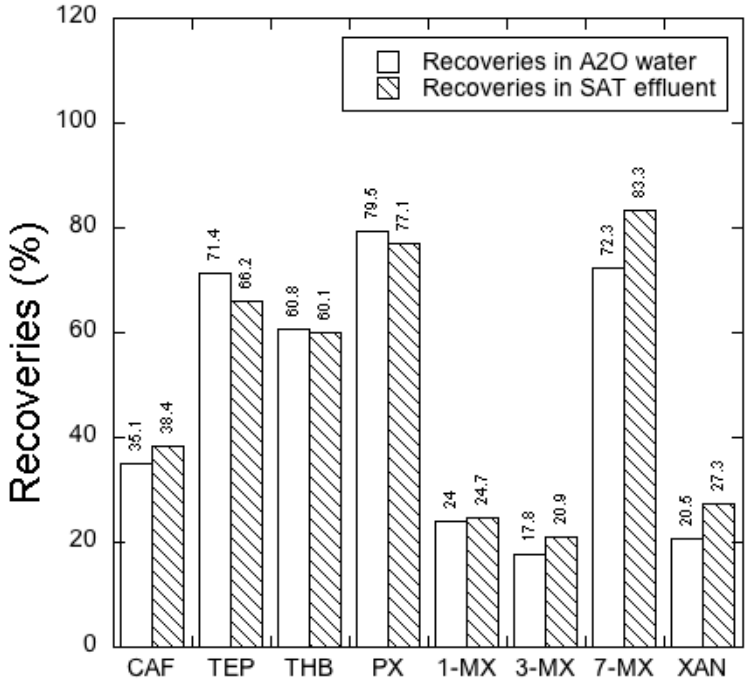


Figure 5.2 –The recoveries of CAF and its TPs in A_2O water and SAT effluent.

5.2.3.4. Numerical simulation

For simulating the demethylation of CAF in SAT and AS treatment, numerical simulation was performed by using the Copasi program in the transformation experiment with the addition of CAF, and the first-order reaction kinetic model was conducted in the demethylation of CAF. Considering the rate constants of transformation, in this simulation, the transformation routes of CAF in SAT and AS treatment were compared. The first order rate constants of demethylation (k) were calculated by the formula as follows:

$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = k[C]$$

Where C is the concentration of target compounds (i.e., CAF, TEP, PX and so on); t is the reaction time.

5.3. Results

5.3.1. Comparison of CAF TPs between SAT and AS system

5.3.1.1. Biodegradation of CAF TPs in SAT and AS treatment

Consecutive demethylation was expected in the transformation of CAF. Thus, the degradation rate of each TP was evaluated in details by spiking 1 μg/L of TP (Figure 5.3).

In SAT and SBR, the concentration of CAF TPs decreased readily. Hillebrand et al.[7] reported that the concentration of CAF, PX, THB, TEP, 1-MX, and 3-MX rapidly decreased in the water samples from WWTPs. From the slopes of time-concentration profiles, the degradation rates of CAF TPs in these two treatments were determined as shown in Table 5.7. The degradation rates of CAF TPs in SAT were lower than that in SBR. This was in agreement with the observation that SBR degraded biodegradable compounds rapidly as discussed in Chapter 3.

In SAT, the order of degradation rate was THB > 1-MX > TEP > PX > 3-MX > 7-MX. That is, THB and TEP were degraded rapidly among dimethylxanthines (THB, TEP, and PX), and 1-MX was degraded rapidly among methylxanthines (1-MX, 3-MX, and 7-MX). In SBR, the order of degradation rate was 7-MX > 1-MX > TEP > THB > PX > 3-MX. The degradation of TEP, THB, and PX was fast with similar rate constants. 1-MX and 7-MX were removed rapidly among methylxanthines.

Table 5.7 –Degradation rate constants of CAF TPs in SAT and SBR (Initial concentration: approximately 1 μg/L).

Compounds	1-MX	3-MX	7-MX	TEP	THB	PX
SAT (μg/(L·h))	0.0061	0.0014	0.0004	0.0043	0.0074	0.0024
SBR (μg/(L·h))	0.078	0.029	0.081	0.033	0.031	0.031

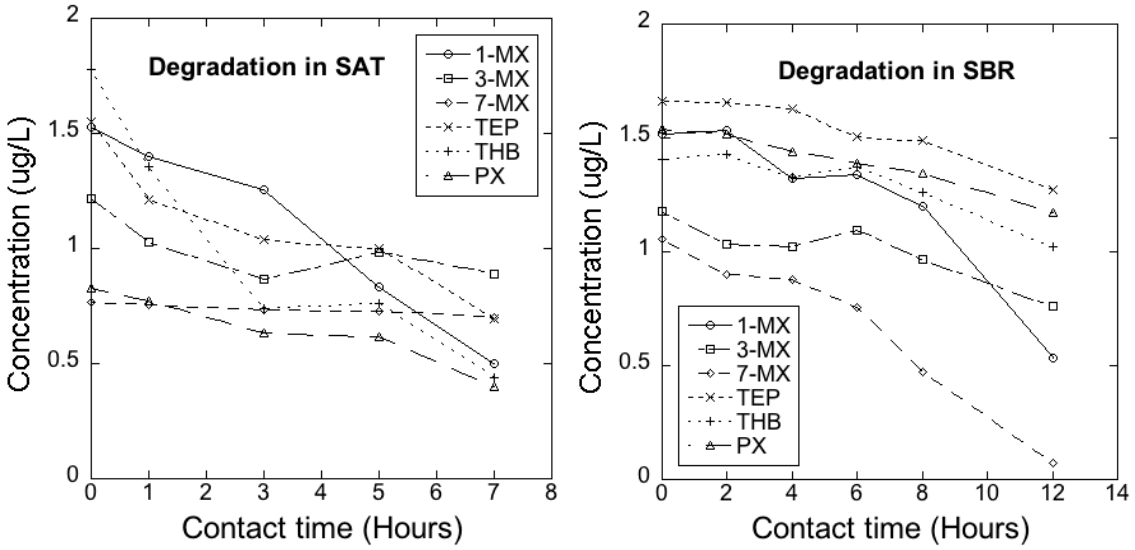


Figure 5.3 –Degradation of CAF TPs (spiking 1 µg/L) in SAT and SBR.

5.3.1.2. Transformation of CAF TPs in SAT and AS treatment

In order to evaluate the transformation pathways of CAF in SBR and SAT, the initial CAF concentration was elevated to 1 µg/L from the ambient concentration (138.4 ng/L and not detected). Normalized time was applied to compare transformation pathways in these two treatments. Normalization formula is shown as follows:

$$Normalized\ time = \frac{t_n}{t_{1/2}}$$

where t is the sampling time and $t_{1/2}$ is the half-life of CAF in each system. Based on the degradation of CAF in both systems, $t_{1/2}$ was 6.6 h in SBR and 3.5 days in SAT. Similar half lives of CAF were observed in the previous studies: Lin et al. [8] reported that $t_{1/2}$ of CAF was 1.5 days in laboratory batch studies with 100 g soil/sediments and 500 mL water; Buerge et al. [9] reported that $t_{1/2}$ of CAF in activated sludge treatment ranged from 0.8 to 5.0 h.

Though the initial concentrations of CAF TPs in the samples of SAT biomass suspension and SBR were different, 1 µg/L of CAF was spiked into these samples to ensure the similar initial concentration of CAF for parallel comparison. The concentration profiles of CAF and its TPs were shown in Figure 5.4.

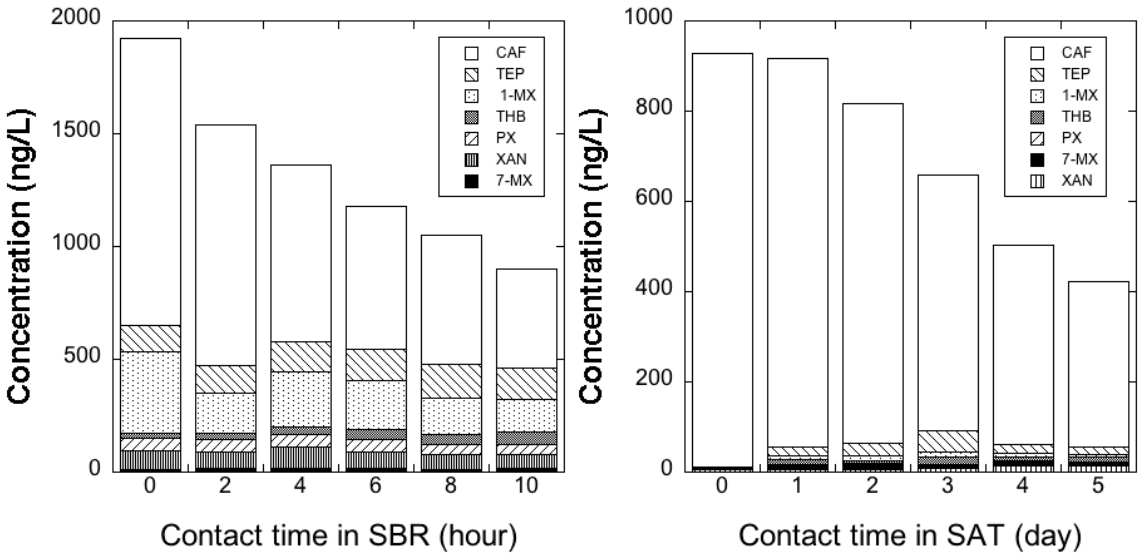
Figure 5.4 shows that CAF was degraded readily in both SBR and SAT, but simulated concentrations of CAF decreased more slowly than measured concentrations of CAF in these treatments, which could be attributed to other transformation such C-8 hydroxylation of CAF [10]. For the first-step demethylation, the concentration of TEP increased in the initial phase (From 120.2 ng/L to a maximum of 149.2 ng/L at normalized time of 1.2), and the concentration of 1-MX decreased in the following phase in SBR. A similar TEP time-concentration profile was observed in SAT biomass suspension (maximum of 47.5 ng/L at normalized time of 0.9). The concentration of THB

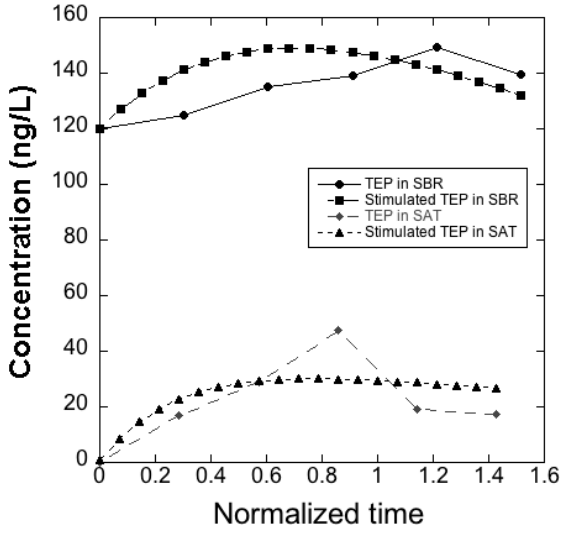
increased steadily in SBR (from 22.2 to 56.2 ng/L) and in the initial phase (normalized time of 0-1.1, 28.9 ng/L) of SAT biomass suspension. Simulated concentration profiles of THB indicated that THB was formed by transformation of CAF and was further degraded. However, while the concentration of PX decreased in SBR, and was kept in a low concentration level (below 3 ng/L) in SAT biomass suspension.

For the second-step demethylation, the concentration of 1-MX fluctuated in SBR (360.8 - 141.0 ng/L), and increased in SAT biomass suspension (from N.D. to 26.9 ng/L). In addition, the concentration of 7-MX fluctuated in the range of 12.2 and 19.2 ng/L in SBR, and increased from N.D. to 7.4 ng/L in SAT. 3-MX was not detected in both systems.

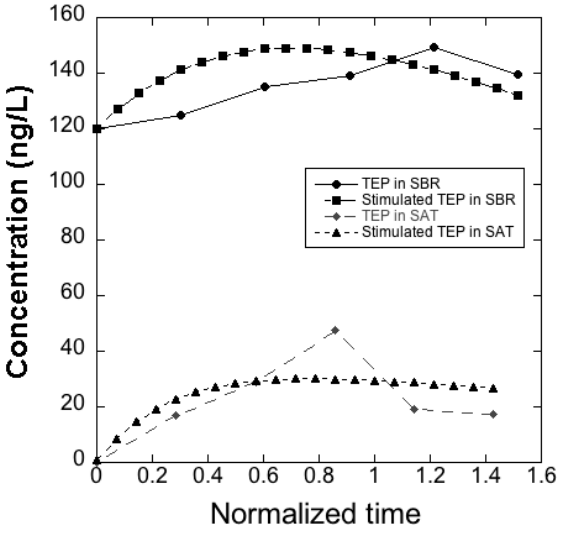
For the third-step demethylation, the concentration of XAN increased readily in both SBR (191.0 ng/L) and SAT (68.7 ng/L). XAN was moderately biodegradable in SBR as discussed in the following part. However, simulated concentration of XAN in SBR decreased after normalized time of 0.8.

In summary, TEP, THB, PX, 1-MX, 7-MX, and XAN were formed during the degradation of CAF in both SAT and SBR.

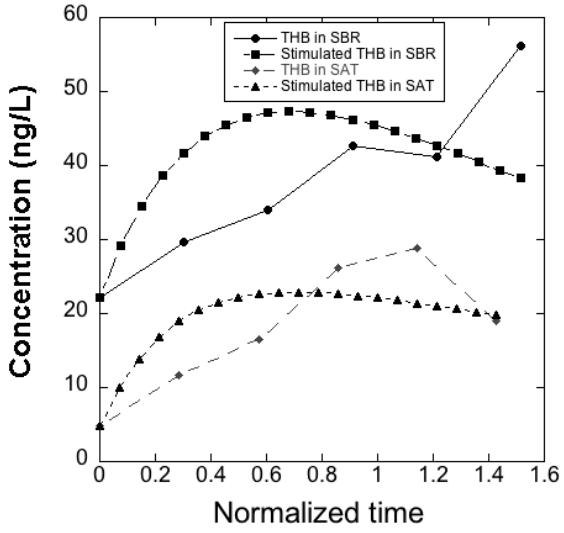




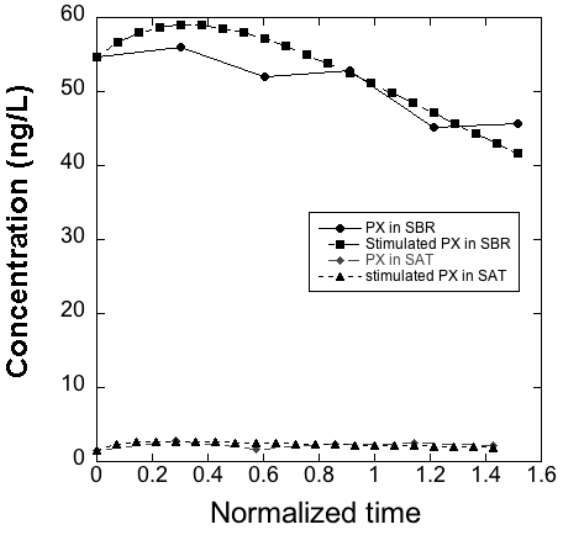
(a) CAF



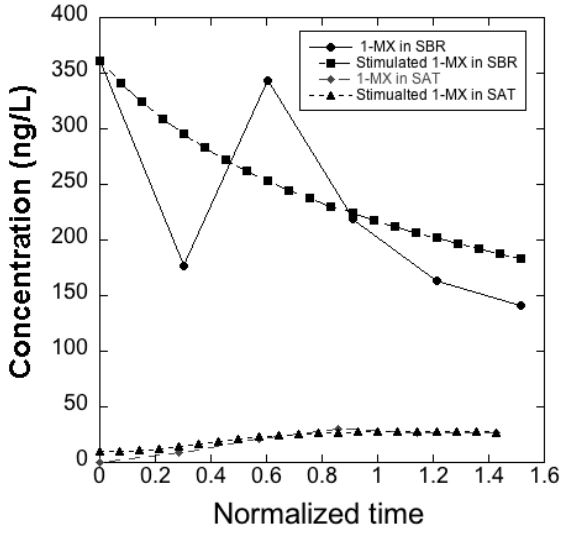
(b) TEP



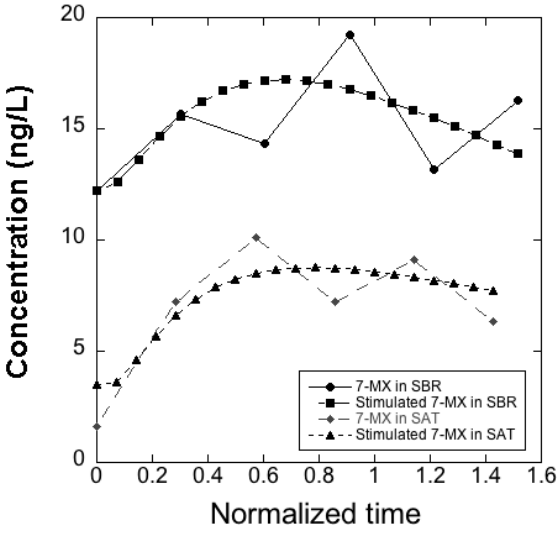
(c) THB



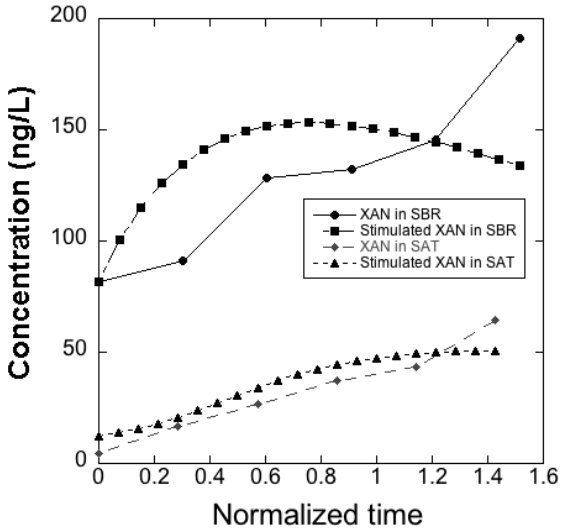
(d) PX



(e) 1-MX



(f) 7-MX



(g) XAN

Figure 5.4 –Concentration trends of CAF and CAF TPs in SBR and SAT biomass suspension.

5.3.1.3. Occurrence of CAF TPs in SAT and AS treatment

The concentrations of CAF and its TPs (PX, THB, TEP, 1-MX, 3-MX, 7-MX and XAN) in A_2O water, SBR effluent, effluents from short lab-scale columns (WGS-4H and WGS-8H) and medium lab-scale columns (WGS-7) are shown in Figure 5.5. In A_2O water, TEP, THB, PX, 1-MX, 7-MX, and XAN were detected at 152.9, 37.6, 27.7, 296.6, 9.2, and 120.3 ng/L, respectively. That is, the concentrations of TEP, 1-MX, and XAN

were higher than their parent compound, CAF (138.4 ng/L). In SBR effluent, the concentrations of CAF, TEP, THB, PX, 1-MX, and XAN were 87.1, 103.7, 23.9, 10.5, 104.3, and 60.6 ng/L, respectively, and 3-MX and 7-MX were not detected. In SAT columns, the concentrations of TEP increased in WGS-4H and WGS-8H (167.3 and 169.1 ng/L, respectively), but decreased in WGS-7 (54.3 ng/L). For other CAF TPs, they were readily removed in WGS columns. With extending HRT in WGS columns, the removal efficiencies of CAF and its TPs were improved.

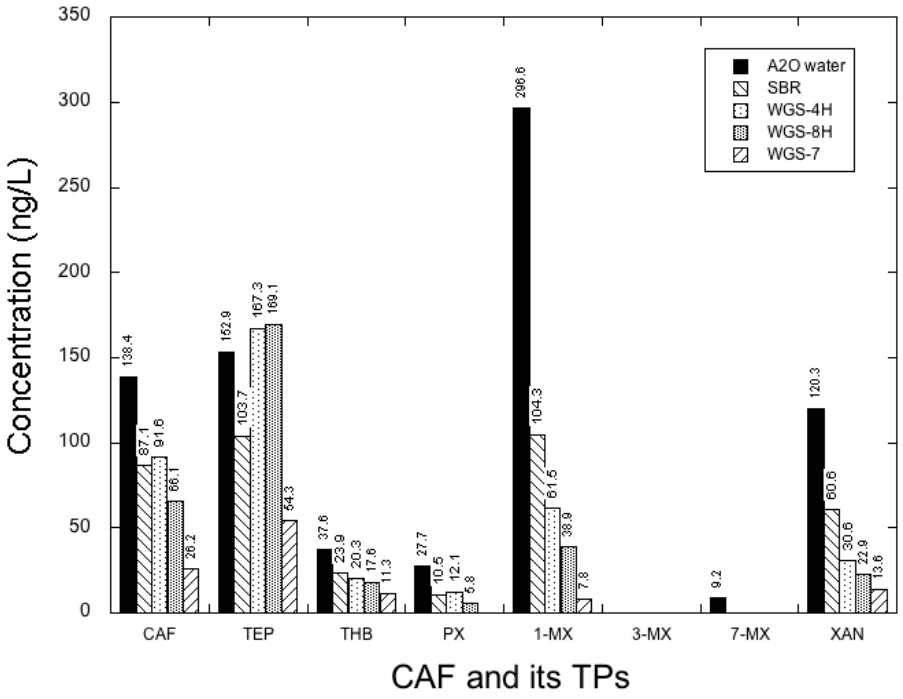


Figure 5.5 –The concentration of CAF and its TPs in A_2O water, SBR effluent, and SAT effluents (n=3).

5.3.2. Formation of profen TPs between SAT and AS system

Profens were easily biodegradable according to the results in Chapter 3. The MRM conditions for the potential TPs of IBP, FNP, NPX, and KTP were proposed as shown in Table 5.4. For biodegradation experiments of profens, the peaks of TPs should meet the conditions as discussed in 5.2.3.2. The increase of peak areas of compounds with predicted pairs of Q1/Q3 was evaluated as the formation of TPs.

In the following figures, the ratio of P_t to P_0 was used for the growth of peak areas. P_t is TP peak area at each sampling time, and P_0 is the initial TP peak area.

5.3.2.1. TPs of IBP in AS treatment and SAT

The molecular weight (MW) of IBP is 206.3 g/mol, and Q1/Q3 pair of IBP in the negative mode is 205.1/161.1. With respect to the MW and Q1/Q3 pairs of IBP, Q1/Q3 pairs of possible TPs (i.e., the MW of oxidated TP is expected as 221) were observed as

listed in Table 5.8. The Q1/Q3 pairs of 221.1/177.1, indicating a 16 Da increase from IBP, were found at two different retention times (9.25 and 10.03 min). These two TPs were marked as 221.1/177.1 (1) and 221.1/177.1 (2) in Figure 5.6. Q1/Q3 pairs of 237.1/193.1, 233.1/189.1, and 253.1/209.1 indicated 32, 28, and 48 Da increases from IBP. Q1/Q3 pairs of 191.1/147.1 and 203.1/159.1 indicated 14 and 2 Da decreases from IBP.

In addition, the TPs with the Q1/Q3 pair of 221.1/177.1 (increase of 16 Da, increased by 10.7 times (1) and 10.4 times (2), respectively) in SBR developed fastest, and they were assumed as primary TPs in SBR. Fast growth of peaks possessing Q1/Q3 pairs of 221.1/177.1 and 191.1/147.1 in SAT indicated TPs with a 16 Da increase and a 14 Da decrease from IBP were expected as primary TPs in SAT. In summary, TP possessing Q1/Q3 pair of 221.1/177.1 was expected in both AS treatment and SAT, but TP with Q1/Q3 pair of 253.1/209.1 was observed only in SAT.

Table 5.8 –Q1/Q3 pairs of IBP TPs found in SBR and SAT.

	SBR	SAT
Q1/Q3 pairs	221.1/177.1 237.1/193.1 191.1/147.1 203.1/159.1 233.1/189.1	221.1/177.1 253.1/209.1 191.1/147.1

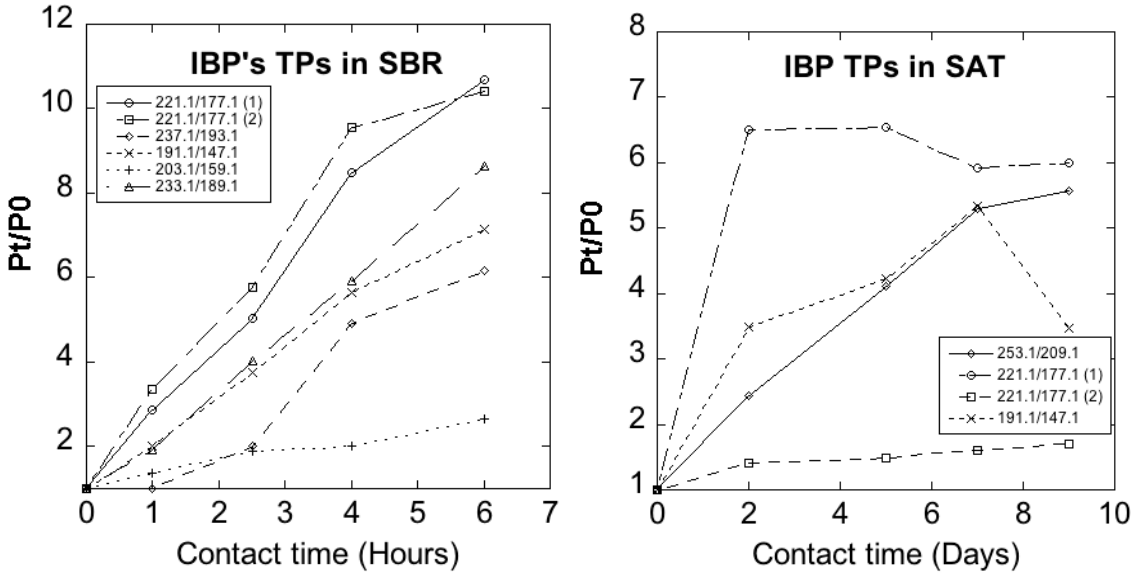


Figure 5.6 –Relative peak areas of IBP TPs found in SBR and SAT with spiking IBP (100 µg/L).

Two TPs with a 16 Da increase from IBP in Table 5.4 indicated the oxidation of IBP. From the fragments of IBP (Figure 5.7), the structures of IBP TPs were estimated. For IBP TPs with retention time of 9.25 min (hereinafter called IBP-TP1), fragments ions were 177.0, 133.1, and 119.1 in the EPI mode. Fragment of 177.0 indicated a loss of 44 Da from MW of IBP-TP1. This suggested the existence of carboxyl group in its chemical structure. IBP possesses fragment of 133 in its chemical structure, and fragment ion of 133 was detected in IBP-TP1. This suggested that IBP-TP1 possessed the same fragment as IBP, indicating hydroxyl group cannot be attached to the fragment of 133. Thus, IBP-TP1 was estimated to be 2-hydroxy-2-(4-isobutylphenyl) propanoic acid (Figure 5.8).

For the IBP TP with the Q1/Q3 pair of 221.1/177.1 and the retention time of 10.03 min (hereinafter called IBP-TP2), fragment ions in the EPI mode were 191.0, 177.1, 175.1, 165.1, and 161.1. Fragment of 177.1 also indicated the existence of carboxylic acid as discussed above. Fragment of 191.0 indicated a 30 Da decrease from the MW of IBP-TP2. Thus, two methyl groups existed in the chemical structure of IBP-TP2. Fragment of 175 indicated a loss of two methyl groups and one hydroxyl group from the chemical structure of IBP-TP2. Thus, IBP-TP2 was estimated to be 2-(4-(2-hydroxy-2-methylpropyl)phenyl) propanoic acid (2-hydroxyibuprofen) (Figure 5.8).

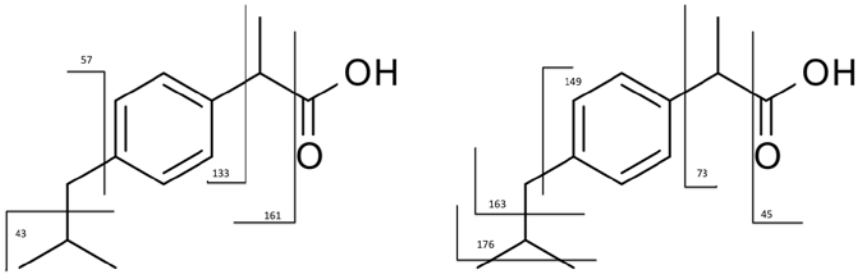


Figure 5.7 –The fragments of IBP.

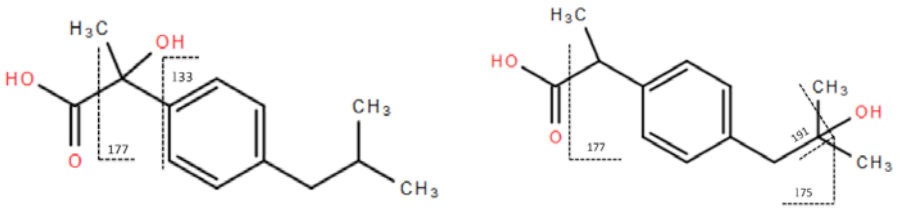


Figure 5.8 –The chemical structure of IBP-TP1 (left) and IBP-TP2 (right).

5.3.2.2. TPs of KTP in AS treatment and SAT

The MW of KTP is 254.281 and Q1/Q3 pair of KTP in the negative mode is 252.9/209.0. As listed in Table 5.9, Q1/Q3 pairs of 428.9/385.0, 300.9/209.0, 268.9/225.0, and 254.9/211.0 were observed indicating 176, 48, 16, and 2 Da increases from KTP, respectively. The Q1/Q3 pairs of 250.9/207.0 and 238.9/195.0 indicated 2 and 14 Da decreases from KTP, respectively. Among observed KTP TPs, TP with Q1/Q3 of 254.9/211.0 increased most rapidly in both SBR and SAT (increased 23.7 and 10.0 times, respectively). Besides Q1/Q3 pair of 254.9/211.0, TPs with Q1/Q3 pairs of 268.9/225.0 and 238.9/195.0 were expected in SAT. In summary, AS treatment and SAT were similar on the formation of TPs possessing Q1/Q3 pair of 254.9/211.0, and more TPs were expected in SAT.

Table 5.9 –Q1/Q3 pairs of KTP TPs found in SBR and SAT.

	SBR	SAT
Q1/Q3 pairs	254.9/211.0 250.9/209.0	268.9/225.0 300.9/209.0 254.9/211.0 238.9/195.0 428.9/385.0

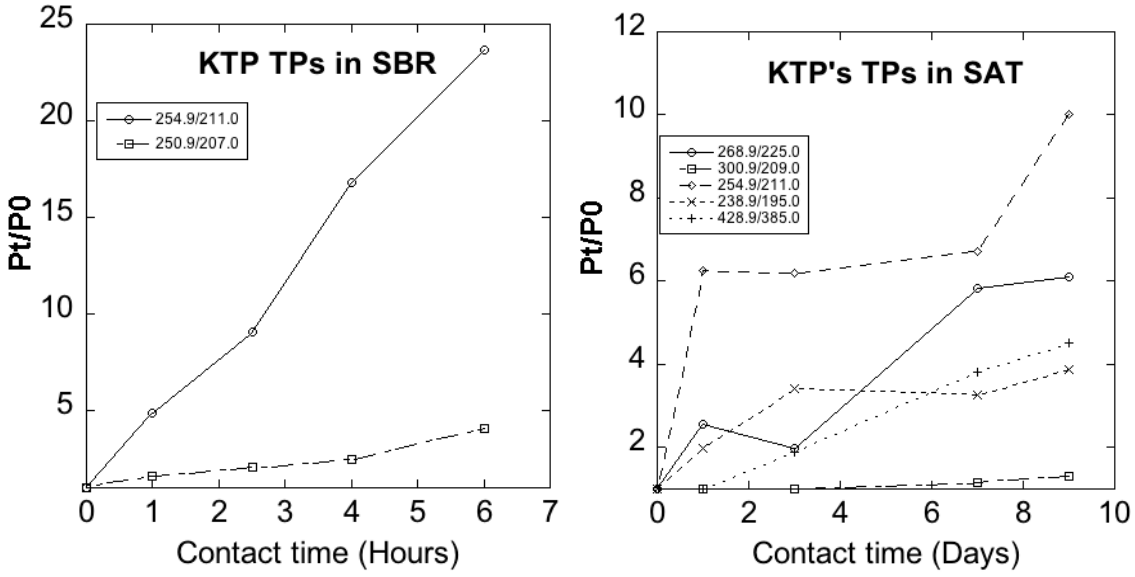


Figure 5.9 –Relative peak areas of KTP TPs found in SBR and SAT with spiking KTP (100 µg/L).

Q1/Q3 pair of 254.9/211.0 was observed in both AS treatment and SAT, so dehydrogenation was expected in both systems, and dihydro KTP was considered as a primary TP of KTP. In A_2O water, the concentration of dihydro KTP in A_2O water was 75.8 ng/L. In the effluents of SBR and WGS-7 column, the concentrations of dihydro KTP were 15.9 ng/L, and not detected, respectively.

In order to confirm the hypothesis of that KTP was transformed into dihydro KTP, the occurrences of dihydro KTP were evaluated in SBR and SAT by adding 1 µg/L of KTP (Figure 5.10). Normalization formula was shown as mentioned in 5.3.1.2. Where *t* is the sampling time and $t_{1/2}$ is the half-life of KTP in each system as described in Table 3.12 and Table 3.13.

In the initial phase, the concentration of dihydro KTP increased, and then after 5 h in SBR and 4 days in SAT, the concentration decreased in the following phase. These suggested that dihydro KTP was biodegradable in both SAT and AS treatment, which was in agreement with the work of Quintana et al. [3].

In summary, dihydro KTP was detected in A_2O water at relatively high concentration, and it is biodegradable in both AS treatment and SAT.

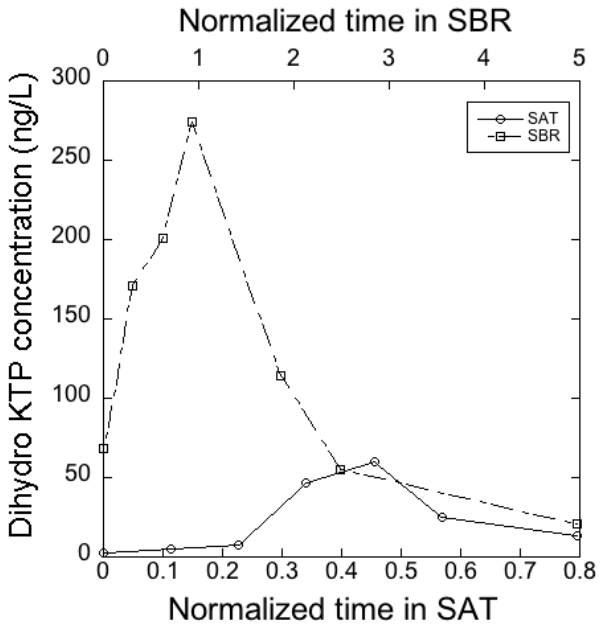


Figure 5.10 –The concentration of dihydro KTP in SAT and SBR.

5.3.2.3. TPs of NPX in AS treatment and SAT

The MW of NPX is 230.259 g/mol and Q1/Q3 pair of NPX in the negative mode was 228.9/185.0. As shown in Table 5.10, Q1/Q3 pairs of 276.8/233.0, 260.8/185.0, 256.9/213.0, 246.9/185.0, 244.9/201.0, and 242.9/199.0 were found as possible TPs indicating 48, 32, 28, 18, 16, and 14 Da increases from NPX, respectively. Q1/Q3 pair of 214.8/171.0 indicated a 14 Da decrease from NPX, respectively. In SBR, TPs with

Q1/Q3 pairs of 244.9/201.0 and 214.8/171.0 increased significantly (more than 10 times). In SAT, TPs with Q1/Q3 pairs of 246.9/185.0 and 214.8/171.0 increased significantly (more than 6 times).

In summary, TP with a Q1/Q3 pair of 214.8/171.0 was formed in both AS treatment and SAT. However, the TP with a Q1/Q3 pair of 244.9/201.0 was found in AS treatment, and the TP with a Q1/Q3 pair of 246.9/185.0 was found in SAT.

Table 5.10 –Q1/Q3 pairs of NPX TPs found in SBR and SAT.

	SBR	SAT
Q1/Q3 pairs	244.9/201.0 260.8/185.0 276.8/233.0 246.9/185.0 214.8/171.0 242.9/199.0	244.9/201.0 276.8/233.0 214.8/171.0 256.9/213.0

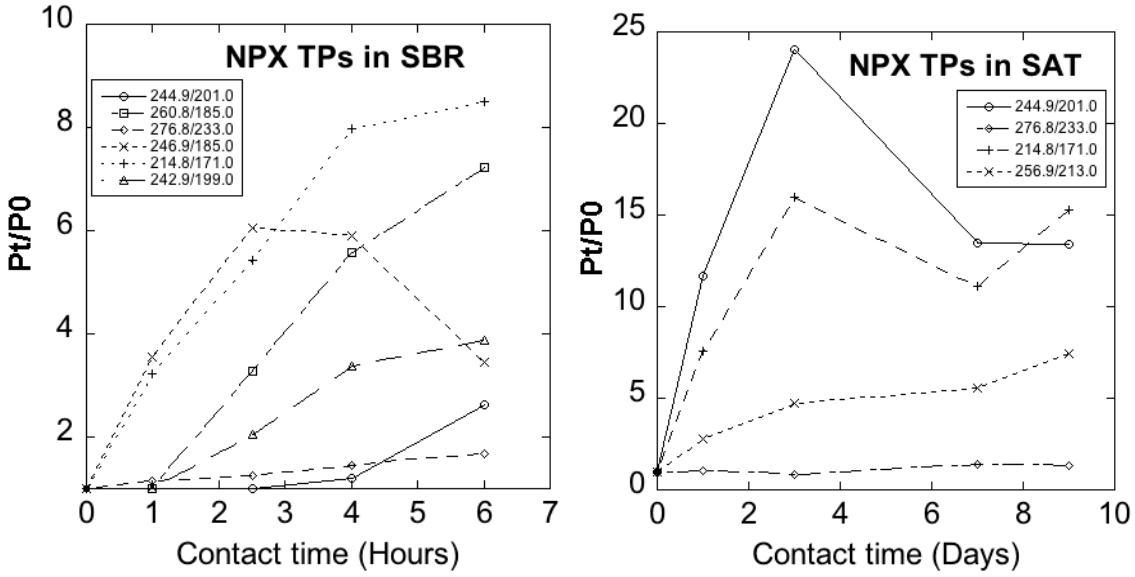


Figure 5.11 –Relative peak areas of NPX TPs found in SBR and SAT with spiking NPX (100 µg/L).

5.3.2.4. TPs of FNP in AS treatment and SAT

The MW of FNP is 242.26 g/mol and Q1/Q3 pair of FNP in the negative mode was 240.9/196.9. As shown in Table 5.11, Q1/Q3 pairs of 272.9/92.8, 268.9/224.9, 258.9/214.9, 256.9/212.9, 254.9/210.9, and 242.9/198.9 indicating 32, 28, 18, 16, 14, and 2 Da increases from FNP were found. Q1/Q3 pairs of 226.9/182.9 and 238.8/194.9

indicated 14 and 2 Da decreases from FNP. Among observed FNP TPs, TP with Q1/Q3 pair of 256.9/212.9 increased most rapidly in SBR, followed by TPs with Q1/Q3 pairs of 242.9/198.9 and 254.9/210.9. In SAT, TP with Q1/Q3 pair of 238.8/194.9 increased most rapidly in SAT, followed by TPs with Q1/Q3 pairs of 256.9/212.9 and 258.9/214.9.

In summary, Q1/Q3 pair of 256.9/212.9 was formed in both AS treatment and SAT. The TP with a Q1/Q3 pair of 256.9/212.9 was the primary TP in SBR as well as TP with a Q1/Q3 pair of 238.8/194.8 in SAT.

Table 5.11 –Q1/Q3 pairs of FNP TPs found in SBR and SAT.

Treatment	SBR	SAT
Q1/Q3 pairs	256.9/212.9 242.9/198.9 254.9/210.9 268.9/224.9 258.9/214.9	256.9/212.9 258.9/214.9 226.9/182.9 238.8/194.9 272.9/92.8

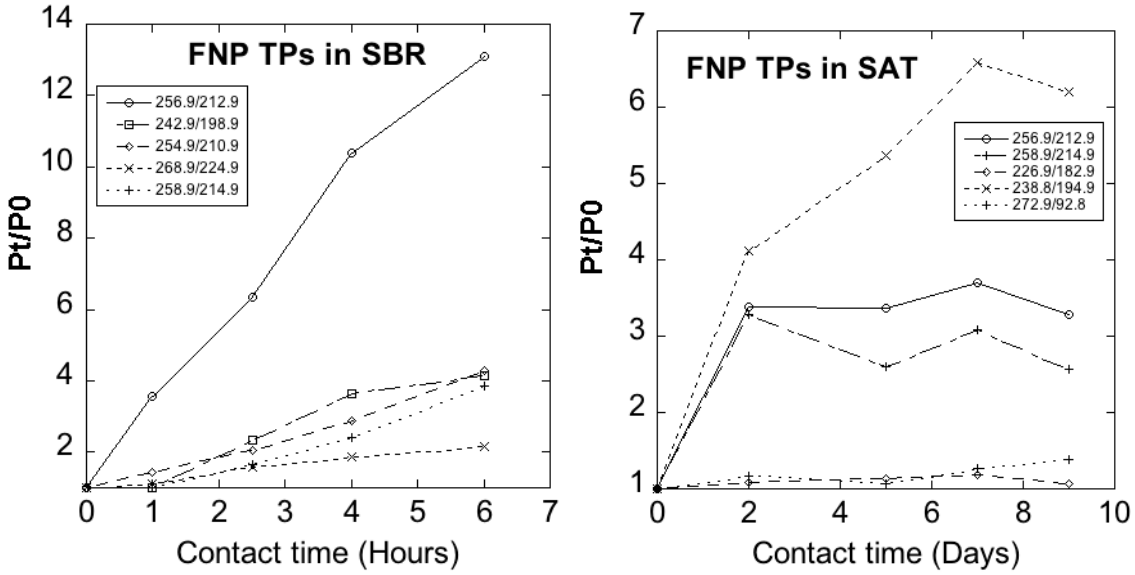


Figure 5.12 –Relative peak areas of FNP TPs found in SBR and SAT with spiking FNP (100 µg/L).

5.4. Discussion

5.4.1. Comparison of CAF TPs between SAT and AS system

5.4.1.1. Transformation of CAF

1) CAF demethylation in SAT and AS treatment

For understanding the pathway of CAF demethylation, the demethylation rate constants were evaluated. The first-order rate constants of demethylation from CAF to TEP, THB, and PX in SBR were 0.03, 0.02, and 0.02 h^{-1}, respectively. This indicated that TEP, THB, and PX were expected as main CAF TPs in SBR. The first-order rate constants of demethylation in SAT from CAF to TEP, THB, and PX were 0.04, 0.03, and 0.01 d^{-1}, respectively. This indicates that TEP and THB were primary TPs of CAF in SAT but PX is not primary TPs of CAF in SAT. Biological demethylation was attributed to the enzyme of biological communities [11-13]. Rich biological communities including fungal communities and bacteria were expected in activated sludge [14, 15] as well as in soil [16, 17]. For the degradation pathway of CAF, Gokulakrishnan et al. [18] mentioned that the types of microorganisms contributed to degradation products of CAF. For example, TEP were found as the initial degradation product of CAF in fungi [19]; CAF is initially converted into THB and PX in parallel by demethylases in bacteria (*Pseudomonas*)[20]. For the primary TPs, THB and TEP with small amounts of PX were considered as the major TPs in the first step [21-23]. These were in agreement with the pathway discussed above.

After first demethylation, TEP, THB and PX were further demethylated. 1-MX, 3-MX, and 7-MX were considered as intermediates of the secondary demethylation in bacteria[18]. Hakil et al.[19] mentioned that THB, 7-MX, and XAN are the major intermediaries in the degradation of CAF in the presence of fungi. Yu et al. [22] found that *Pseudomonas putida* CBB5, which was isolated from soil by enrichment on CAF, could degrade TEP into 1-MX and 3-MX. Summers et al.[24] demonstrated that N3-demethylation of THB and N1-demethylation of PX were expected to form 7-MX. However, 3-MX was not detected in this experiment. 1-MX and 7-MX were the secondary TPs in SAT and AS treatment. In Figure 5.3, the concentration of 7-MX increased in SBR and SAT. For 1-MX, its concentration increased in SAT, but decreased in SBR. For the secondary demethylation, 1-MX was formed from the demethylation of TEP and PX, and 7-MX was formed from the demethylation of THB and PX. However, the first-order rate constant of PX demethylation to 1-MX (k_6) was lower than that to 7-MX (k_7). This indicated that PX was more easily degraded into 7-MX than 1-MX.

For the tertiary demethylation, the concentration of XAN increased both in SAT and SBR, and the trends of XAN between SAT and SBR were similar in the normalized time monitored. Dash et al. [25] found that *Pseudomonas* sp degraded methylxanthines including 7-MX into XAN. In the SBR degradation model, the first-order rate constant of 1-MX degradation (k_8, 0.18 h^{-1}) was much lower than that of 7-MX degradation (k_9, 1.67 1/h). These indicated that XAN was the TPs of the tertiary demethylation of CAF, and more easily formed by the demethylation of 7-MX than the demethylation of 1-MX in

SBR. In SAT degradation model, XAN was formed by the demethylation of 1-MX and 7-MX. Considering the rate constants, the primary route of CAF demethylation in AS treatment was CAF, THB, 7-MX, and XAN, but the primary route of CAF demethylation in SAT was CAF, TEP, 1-MX, and XAN. The difference in the transformation pathways could be attributed to the diversity of CAF-degrading bacteria, primarily *Pseudomonas* in soil by enrichment on CAF [22, 24] .

In the degradation experiment of CAF TPs, In SBR, 1-MX and 7-MX were biodegraded more rapidly than TEP, THB, and PX. Liu and Meissner [26] demonstrated that the existence of methyl functional groups in CAF increased its hydrophobicity and decreased its biodegradability. Furthermore, Arnot et al. [27] mentioned that methyl substitutions on the benzene ring contribute to the resistance to biotransformation. Thus, in SBR, increasing number of methyl functional groups decreased the biodegradability of CAF TPs. However, TEP, THB, and PX were biodegraded more rapidly than 3-MX and 7-MX in SAT. These indicated that SAT has the advantage on the degradation of multi-methyl xanthines compared with AS treatment, which explained the result that CAF (1, 3, 7-trimethylxanthines) was more biodegradable in SAT than in AS treatment.

AS treatment and SAT had different pathways of CAF transformation. That is, different biodegradation types were expected between AS treatment and SAT. Thus, it is reasonable to expect the cooperation of SAT and AS treatment for wastewater reclamation.

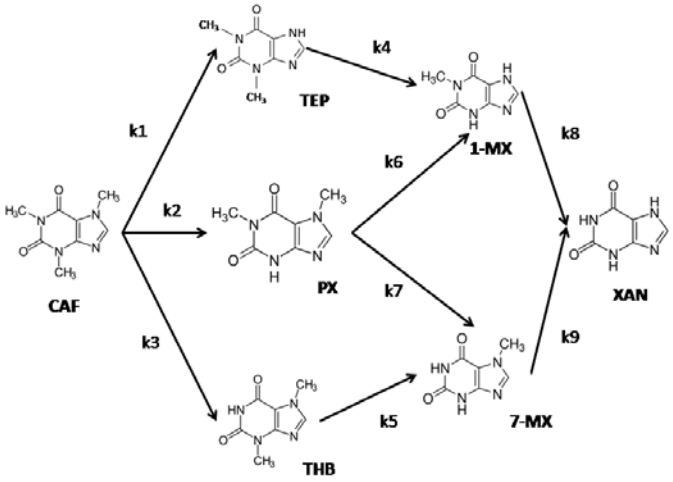


Figure 5.13 –Transformation route of CAF in SAT and AS treatment.

Table 5.12 –The first-order rate constants in SBR and SAT.

the first-order rate constant	SBR(1/h)	SAT (1/d)

k_1	0.03	0.04
k_2	0.02	0.03
k_3	0.02	0.01
k_4	0.17	0.95
k_5	0.36	1.00
k_6	0.09	1.67
k_7	0.25	2.00
k_8	0.18	1.03
k_9	1.67	2.90

2) Occurrence of CAF TPs

In A_2O water, CAF TPs (TEP, THB, PX, 1-MX, 7-MX, and XAN) were detected at relatively high concentration (above 10 ng/L) except 7-MX. Kim et al. [28] reported that TEP was detected at high concentration (0.023 μg/L on average) in the effluent of WWTPs. Nicolardi et al. [29] reported that THB was detected at above 100 ng/L (n = 4) in the effluents of several WWTPs. Nödler et al. [30] reported that 1-MX was detected at 61 ng/L in river samples. These indicated that CAF TPs were widely detected in the effluents of WWTPs, and their risks will be discussed in 5.4.3.

In SBR, the removal efficiencies of 1-MX and XAN were higher than the removal efficiencies of CAF, TEP, THB, and PX. This fitted the former discussion that 1-MX were biodegraded more rapidly than TEP, THB, and PX. Hillebrand et al. [7] reported that 1-MX was biodegraded more rapidly in wastewater effluent than CAF, PX, THB, and TEP. These indicated that AS treatment degraded methylxanthines more rapidly than dimethylxanthines and CAF (trimethylxanthine).

In SAT, the concentration of TEP increased in SAT columns with short HRT (4 h and 8 h), and this was attributed to that CAF was biodegraded into TEP in the initial phase. As discussed above, the removals of CAF and its TPs were improved by extending HRT, and SAT with an HRT of 7 days removed CAF and its TPs effectively. Thus, for the practical purpose, sufficient HRT (7 days) of SAT was necessary to ensure the further biodegradation of TPs.

5.4.2. Profen TPs in SAT and AS system

5.4.2.1. Transformation of profens in SAT and AS treatment

For the transformations of IBP, oxidation (Q1/Q3 pair of 221.1/177.1) indicated that biological hydroxylation was important in the transformations of IBP [31]. In this study, two hydroxyl-IBPs were found as oxidated TPs. Weigel et al.[32] and Zwiener et al. [33] found that hydroxyl-IBP was identified as the major metabolite of IBP in the oxic biofilm reactor. Collado et al. [34] mentioned that 32% of IBP was transformed into hydroxyl-IBP in activated sludge in 24 h. From the perspective of chemical structures (Figure 5.8), IBP-TP1 and IBP-TP2 were hydroxyl-IBPs with different hydroxyl

positions. IBP-TP1 and IBP-TP2 increased similarly in SBR, but IBP-TP1 increased more rapidly than IBP-TP2 in SAT. Furthermore, in SAT, Q1/Q3 pair of 253.1/209.1 (a 48 Da increase) indicated that tri-oxidation was expected as well as oxidation. Murdoch et al. [35] mentioned that tri-hydroxyl-IBP could be a dead-end metabolite, and was observed in activated sludge. In this study, the existence of tri-hydroxyibuprofen indicated successive hydroxylation in SAT. In addition, TP with a 14 Da decrease was found in the transformation of IBP in both SAT and AS treatment. Michael et al. [36] reported that direct demethylation of IBP was observed in parallel reaction with the hydroxylation process. Thus, demethylation was expected in AS treatment and SAT. According to the growth of relative peak areas, hydroxylation was primary transformation process in the biodegradation of IBP in SAT and AS treatment.

For the transformation of KTP, hydrogenation (Q1/Q3 of 254.9/211.0, a 2 Da increase) was important in both SBR and SAT. Benoit et al. [37] found that before dioxygenation of KTP, a reduction of the keto group may occur in mammal's metabolism, and this reduction increases the electron density of KTP, resulting in KTP more reactive towards an electrophilic dioxygenation. For the bacteria degradation, Quintana et al. [3] mentioned that the first step of KTP degradation was the reduction of the keto group, and dihydro-KTP was a metabolite of KTP, which can be degraded further. In this study, dihydro-KTP was detected at A_2O water, and Figure 5.10 indicated that dihydro-KTP was formed from KTP. In addition, hydroxylation of KTP was found in SAT, but not found in SBR. Marco-Urrea et al. [38] mentioned that 2-[3-(4-hydroxylbenzoyl)-phenyl]-propanoic acid was formed from KTP by direct hydroxylation. Furthermore, Skordi et al. [39] reported that 2-[3-(4-hydroxylbenzoyl)-phenyl]-propanoic acid and 2-[3-(3-hydroxybenzoyl)phenyl]-propanoic acid were the metabolites of KTP in human. Thus, biological communities in SAT can degrade KTP by direct hydroxylation besides hydrogenation, and biological oxidation (hydroxylation) of KTP was expected in SAT.

For the transformations of NPX, Q1/Q3 pair of 214.8/171.0 (a 14 Da decrease) indicated demethylation in these two treatments. In human metabolism, NPX was transformed into O-desmethylnaproxen (a demethylated NPX) by the enzymes of Cytochrome P450 (hereinafter called as CYP) [40]. CYP widely existed in bacteria and fungi. For example, White rot fungi has the potential to metabolize xenobiotics by using CYP in a similar way to mammals [41]. Rodríguez-Rodríguez et al. [42] mentioned that *T. versicolor* in activated sludge could degrade micropollutants, and NPX can be catalyzed by laccase and CYP in *T. versicolor*. Thus, O-desmethylnaproxen was expected as TPs in AS treatment and SAT. Hydroxylation (Q1/Q3 of 244.9/201.0, an 18 Da increase) was expected in SBR. Wojcieszyńska et al. [43] suggests that degradation of NPX occurs by its hydroxylation, and He et al. [44] indicated that 7-hydroxynaproxen was a NPX metabolite by *Aspergillus niger*. Hydroxylation contributed to the transformation of NPX in SAT.

For FNP, oxidation was expected in both SAT and AS treatment. hydroxyl-FNP was the main metabolite of FNP in human bodies [45, 46]. Hoffmann et al. [47] mentioned that FNP could be transformed into 4’-hydroxyfenoprofen by river sediment. Hydroxyl-FNP could be a primary TP of FNP in SBR, but dehydrogenated TPs (2 Da decrease) increased more rapidly than hydroxyl-FNP in SAT. Dehydrogenation was an

important biotransformation pathway because of a minor structural modification of the parent compounds [31]. These indicated that dehydrogenation of FNP was expected in SAT, but not in SBR.

As discussed above and the transformation types in Table 5.4, the transformation processes of profens in SAT and SBR are summarized in Table 5.13.

Table 5.13 –Transformation processes of profens in SAT and SBR.

	IBP		FNP		NPX		KTP	
	SBR	SAT	SBR	SAT	SBR	SAT	SBR	SAT
Oxidation	++	+	++	+	+	+++		++
Di-oxidation	+			+	+			
Tri-oxidation		+			+	+		+
Gain of H_2O			+	+	+			
Hydrogenation			+				++++	++
Demethylation	+	+		+	+	+++		+
Dehydrogenation	+			+			+	
Sulfonation								
Methylation			+		+			
Di-methylation	+		+			+		
Glucuronidation								+

From Table 5.13, oxidation and demethylation were important transformations in transformation of profens. Generally, hydroxylation was considered as an important biological oxidation [48]. In SAT, hydroxylation and demethylation contributed to the biodegradation of all selected profens. However, in SBR, hydroxylated KTP, demethylated KTP, and demethylated FNP were not found. Thus, the wide range of transformation process is expected in SAT. For the range of biodegradation in soil, Andreu et al. [49] also demonstrated that pesticides undergo a variety of transformations, and a complex pattern of metabolites was observed in soil.

Generally, biological hydroxylation is attributed to CYP, and hydroxylation of unfunctionalized alkyl groups in hydrocarbons and fatty acids was expected in the biodegradation of micropollutants [50]. In this study, hydroxylation was expected in all cases expect KTP in SBR. Besides selected profens, hydroxylation also contributed to the transformation of flurbiprofen (4-hydroxy flurbiprofen) in microorganisms (*Cunninghamella spp*) [51]. For other micropollutants, Yonetani [52] reported that the hydroxylation of DCF occurred and hydroxylated DCF was detected in A_2O water and SAT effluent. Buisson et al. [53] mentioned that micropollutants such as PCB and 2,4-dichlorophenol (2, 4-DCP) were hydroxylated by bacteria. Schulz et al. [54] also mentioned that hydroxylation was important for the transformation of iopromide. Thus, hydroxylation could be important for the biodegradation of micropollutants. In this study, SAT hydroxylated a wider range compounds than AS treatment. Hydroxylation is often accompanied with the cleavage of ring-structure, resulting in further transformation to CO_2 and H_2O [3, 55]. Thus, a wider range of hydroxylation in SAT than AS treatment was helpful for the removals of micropollutants, not only the parent compounds, but also their TPs. Overall, the cooperation of SAT and AS treatment was available for the control of micropollutants.

Demethylation was an important transformation pathway as discussed in 5.4.1. Furthermore, not only CAF and its TPs, but also profens were transformed by demethylation. For other micropollutants, The prodrug mestranol is converted after administering into 17α-ethinylestradiol by demethylation [56]. Demethylation was expected in soil for degradation of carboxylic compounds [57]. For example, *Nocardia* was able to cause various activities on benzoic acid derivatives, including demethylation [58]. In this study, compared with in AS treatment, active demethylation of profens was expected in SAT.

5.4.3. Chemical risk assessment of TPs

For the transformation of CAF, according to the research of Komori et al. [59], PNEC of CAF in Japan was 5200 ng/L, and MEC/PNEC more than 0.1 needs further survey. In this study, MEC/PNEC of CAF was less than 0.1, but the total concentration of CAF and its TPs was 782.7 ng/L. The environmental risk of CAF TPs was not clear, but their PNECs could be set to similar values as CAF because of similar structure to CAF. Thus, MEC/PNEC of CAF and its TPs were 0.15, which suggested that their environmental risk needed further survey. Similarly, MEC/PNEC of KTP was 6.15 in this study, and its removal was effective in both AS treatment and SAT. However, dihydro-KTP was formed by hydrogenation of KTP, and its concentrations in A_2O water and SBR effluent were 75.8 and 15.9 ng/L, respectively. These indicated that chemical risk assessment of micropollutants should be evaluated on basis of the occurrence of TPs and their parent compounds even their effective removals in AS treatment and SAT.

5.5. Summary

The results of this study demonstrated the consecutive demethylation of CAF between AS treatment and SAT. TEP, THB and PX were the first-step demethylation TPs; 1-MX and 7-MX were the second-step demethylation TPs; XAN was the final demethylation TPs. SAT has the advantage on the degradation of multi-methyl xanthines compared with AS treatment. Furthermore, TEP and THB were primary TPs of CAF demethylation in SAT. Considering the rate constants, the primary route of CAF demethylation in AS treatment was CAF, THB, 7-MX, and XAN, but the primary route of CAF demethylation in SAT was CAF, TEP, 1-MX, and XAN. Combination of SAT and AS treatment enriches the biodegradation of CAF in wastewater reclamation.

TEP, THB, PX, 1-MX, 7-MX, and XAN were detected in A_2O water, and the concentrations of TEP, 1-MX, and XAN were higher than CAF. CAF TPs were biodegradable in both AS treatment and SAT. SAT with sufficient HRT (7 days) degraded CAF and its TPs effectively. However, SAT with a short HRT in had the risk of increasing the concentration of TPs. A sufficient HRT is necessary to control the risk of TPs.

For the transformation of profens, hydroxylation and demethylation contributed to the biodegradation of all selected profens. For primary TPs, two hydroxyl-IBPs, dehydrogenated KTPs, demethylated NPX, and hydroxyl-FNP were expected in SBR. In

addition, hydroxyl-IBPs, demethylated IBP, dehydrogenated KTPs, demethylated NPX, and dehydrogenated FNP were expected in SAT. SAT showed a wider range of hydroxylation and demethylation pathways than AS treatment.

5.6. Reference

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Chapter 6 Conclusions

6.1. Major findings of this study

Micropollutants cause adverse health effects. Wastewater effluent for reclamation should be free from micropollutants to minimize the risk. SAT was an alternative advanced treatment after AS treatment. During SAT, sorption and biodegradation mainly contributed to the removals of micropollutants. Among micropollutants, PPCPs are diverse in chemical properties, so they can be the indicators of micropollutants to evaluate their removal mechanisms in SAT.

With PPCPs as indicator compounds, this study addressed the following issues: 1) the contributions of biodegradation and sorption on the removals of PPCPs, 2) the effects of operating conditions on the removals of PPCPs, and 3) the transformation pathways and transformation processes. The results provided the new insights on the cooperation of SAT and AS treatment. The major findings in each chapter are summarized below:

In Chapter 3, firstly, the occurrences of PPCPs in wastewater effluents were examined. Secondly, biodegradation and sorption in SAT were evaluated by the experiments of sterilized and non-sterilized columns. Thirdly, the effects of biomass on the sorption in SAT were discussed. Fourthly, the differences of biodegradation between SAT and AS treatment were evaluated. The major findings are as follows:

1) Soil in SAT showed strong sorption on micropollutants possessing high K_{oc} values. The existence of biological communities enhanced for the sorption in SAT. In addition, ionic interaction in SAT contributed the removals of compounds having the values of pK_a above the soil pH.

2) Biodegradation played an important role in removals of micropollutants in both AS treatment and SAT. Carboxyl group increases the biodegradability, and amide functional group decreases the biodegradability in both treatments. Biodegradation of halogenated acid and PAH were less efficient in AS treatment than in SAT. AS treatment degraded the biodegradable compounds rapidly, but SAT biodegraded a wider range of compounds than AS treatment.

3) Overall, SAT has the advantage on the sorption of compounds with high K_{oc} value and pK_a above soil pH as well as the further biodegradation on moderately biodegradable compounds. Thus, the combination of AS treatment and SAT could be an effective choice of controlling micropollutants for improving water quality.

The objective of Chapter 4 was to investigate the effects of operating conditions (packing materials, HRT, and saturated condition) in SAT on the removals of PPCPs. Additionally, microbial communities in SAT and AS treatment were discussed by the microbial substrate metabolic patterns. The major findings are as follows.

1) For biodegradation pattern, SAT showed higher biological activities than natural soil because of feeding A_2O water. SAT has a wider biodegradation range than AS treatment. WGS with an HRT of 180 days showed unique biodegradation compared with other SAT columns.

2) High TOC and CEC enhanced the removals of antibiotics in SAT. Packing materials showed little influence on the biodegradation characters in SAT.

3) In the long-term operation, for most PPCPs, their removals were sufficiently high in WGS column with an HRT of 7 days. Extending HRT from 7 days was helpful for the removals of SMETH, ATP, CRT and ACEAM in SAT. In the short-term operation, removal efficiencies of biodegradable PPCPs were improved by extending HRT.

4) For vadose condition, the removals of most selected PPCPs were similar under vadose and saturated conditions, but SMETH and CRT were effectively removed under vadose condition, which was attributed sustainable aerobic condition in the whole column for their biodegradation.

In Chapter 5, the transformation pathway of CAF and the occurrence of CAF TPs in AS and SAT were evaluated. Moreover, the transformation processes of profens were discussed. The major findings are summarized as follows:

1) The results of this study demonstrated the consecutive demethylation of CAF between AS treatment and SAT. TEP, THB and PX were the first-step demethylation TPs; 1-MX and 7-MX were the second-step demethylation TPs; XAN was the final demethylation TPs. SAT has the advantage on the degradation of multi-methyl xanthines compared with AS treatment. Furthermore, TEP and THB were primary TPs of CAF demethylation in SAT. Considering the rate constants, the primary route of CAF demethylation in AS treatment was CAF, THB, 7-MX, and XAN, but the primary route of CAF demethylation in SAT was CAF, TEP, 1-MX, and XAN.

2) TEP, THB, PX, 1-MX, 7-MX, and XAN were detected in A_2O water, and the concentrations of TEP, 1-MX, and XAN were higher than CAF. CAF TPs were biodegradable in both AS treatment and SAT. SAT with a long HRT degraded CAF and its TPs more effectively. However, the short HRT in SAT had the risk on increasing TPs.

3) For the transformation of profens, hydroxylation and demethylation contributed to the biodegradation of all selected profens. For primary TPs, two hydroxyl-IBPs, dehydrogenated KTPs, demethylated NPX, and hydroxyl-FNP were expected in SBR. In addition, hydroxyl-IBPs, demethylated IBP, dehydrogenated KTPs, demethylated NPX, and dehydrogenated FNP were expected in SAT. It is concluded that SAT has a wider range of hydroxylation and demethylation pathways than AS treatment.

6.2. Further Research

To deepen the understanding of removal mechanisms in SAT, several challenging researches should be carried on in future as follows:

1) Not only PPCPs, a wider range of micropollutants with different physical and chemical properties should be evaluated to establish the quantitative structure activity relationship (QMRA) of micropollutants in SAT.

2) Though SAT can eliminate most PPCPs effectively, amide PPCPs were still resistant in SAT. This indicated that SAT still has a limitation on controlling micropollutants. For the achievement of high-level water quality, the cooperation of SAT and other advanced treatments (i.e., ozone and advanced oxidation process (AOP)) should be considered.

3. TPs of CAF and profens during SAT have been evaluated. However, in this study, the monitoring and estimation of TPs were done by standard compounds and the possible reactions. In the future, unknown transformation pathways and unknown TPs should be evaluated. With a wider range of parent compounds than this study, transformation pathways of biodegradable compounds in treatment should be supported with respect to physical-chemical properties.