Consumption-based estimation of discharge of human-used antibiotics from sewage treatment plants (STPs) to the aquatic environment and risk assessment

2021

MIAO JIA

Consumption-based estimation of discharge of human-used antibiotics from sewage treatment plants (STPs) to the aquatic environment and risk assessment
(消費量をもとにしたヒト用抗生物質の下水処理場から水環境への流出量の推定とリスク評価)

MIAO JIA

A Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Engineering

Department of Urban and Environmental Engineering
Graduate School of Engineering
Kyoto University
Kyoto, Japan

2021

ABSTRACT

Since the massive and continuous usage of antibiotics in our everyday life, it has raised worldwide concerns due to the ecotoxicity in the aquatic environment and development of antibiotic resistant. For the further risk assessment on the antibiotics in the aquatic environment, it is essential to evaluated the discharge of antibiotics from the sewage treatment plants (STPs). The occurrences of antibiotics exhibit great variability influenced by several factors, such as types, locations, seasons and so on. Moreover, monitoring of antibiotics is restricted by the available analytical methods in laboratories, cost and time consumption. Therefore, estimation approach becomes a promising way. Because the discharge of STPs is considered as the main route for human-used antibiotics entering into the aquatic environment, to evaluate the discharge of antibiotics from STPs, it involved two main parts to establish the predictive models: I) to predict the antibiotics concentrations in the sewage influent based on the consumption volume, wastewater production per inhabitant and service population of target STPs; II) to predict the concentration of antibiotics in the STPs effluent based on the removal performance of antibiotics in the STPs. Although many predictive models developed by previous researchers, there is presently no integrate of these models to predict the release of antibiotics from consumption data due to the various concepts. Therefore, it is essential to propose the integrate models suitable for different processes. Previous risk assessment work of antibiotic residues only considered the ecotoxicity, it is urgent to involve the potential risk on the selection and development of antibiotic resistance for antibiotic residues, based on which to propose the discharge limits of antibiotics. Therefore, in this study, two parts of predictive models were established and evaluated by the measured data, the possible strategy was obtained for each model. Risk assessment was carried out for estimated discharge of antibiotics on the perspectives of both environmental and human health, the discharge limit of individual antibiotic from the STPs and the possible strategies for the reduction of released antibiotics above the discharge limit were proposed. The findings obtained in each Chapter are summarized as below.

Firstly, the predictive model based on consumption volumes of human-used antibiotics from two databases (shipping and prescription) had been applied to estimate the sewage influent concentrations of selected antibiotics in two STPs which located on different prefectures, and monitoring data were used to evaluate the accuracy of this predicting equation. In two STPs, among the 9 detected antibiotics, 7 have a predicted environmental concentration in sewage influent ($PECs_{inf}$) calculated by national shipping volume greater than the corresponding measured environmental concentration in sewage influent ($MECs_{inf}$), and 6 have a $PECs_{inf}$ calculated based on national/regional prescription volume greater than the corresponding $MECs_{inf}$. The $PECs_{inf}$ on the basis of prescription volumes are closer to $MECs_{inf}$ than those on the basis of shipping volume, but the predicted concentrations of azithromycin based on the prescription volumes were unacceptably low. There were positive correlations between national shipping and national/regional prescription databases (correlation efficient $r > 0.70$). Therefore, it is possible to use the national shipping volumes to calculate the predicted concentrations when the outliers existed based on the regional prescription data of target compounds, but the predicted concentrations would be somewhat higher than those on the basis of national shipping data. The strategy in this part could be obtained: the $PECs_{inf}$ calculated by the regional prescription data would be applied for further estimation, for the outliers, the consumption volume could be revised on the basis of national shipping volumes.

Secondly, the batch experiments were carried out to study the adsorption and biodegradation performance of target antibiotics in activated sludge from AAO, CAS and MBR system of the two target STPs for the further estimation on the removal in target STPs. For the sorption distribution coefficients (K_d), the empirical predictive model was established and evaluated by the measured data. The biodegradation of target antibiotics was estimated by two kinetics: 1) first-order kinetics; 2) separately characterization on AOB co-metabolic kinetics (zero-order kinetics) and heterotroph biodegradation kinetics (first-order kinetics). All β-lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), sulfamethoxazole and trimethoprim were classified as lowly sorptive ($\log K_d < 2$), clarithromycin and azithromycin were classified as moderately sorptive ($2 \leq \log K_d < 3$), and levofloxacin was the only

compound classified as highly sorptive (log K_d > 3) in this study. The predictive model of sorption distribution coefficient in this study was established and would be applied in further estimation in Chapter V: log K_{oc}= 0.63 log K_{ow} + 1.15, $K_d = f_{oc}K_{oc}$, f_{oc}=0.531, which generally shows good prediction for most compounds with RMSE=0.47. However, the sorption distribution coefficients of levofloxacin were underestimated in all predictive models based on the log K_{ow} values, then the experimental data of levofloxacin would be applied in the further estimation. The first-order kinetic constants (k_{bio}, $L \cdot gVSS^{-1} \cdot d^{-1}$) were estimated on the biodegradation of target antibiotics in the activated sludge from three different redox conditions in STP A_AAO, and aerobic condition in STP B_CAS and STP B_MBR. B-lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin) were highly ($1 < k_{bio} < 5$) or very highly biodegradable ($k_{bio} > 5$) under three redox conditions and all sludge sources. Sulfamethoxazole and levofloxacin were hardly biodegradable under all redox conditions and sludge sources (k_{bio} <0.5 L/gVSS-d). Clarithromycin was highly biodegradable in STP B_MBR and STP A_AAO sludge under aerobic condition, while it was moderately biodegradable in STP B_CAS sludge and hardly biodegradable in STP A_AAO sludge under anoxic and anaerobic condition. Azithromycin was highly biodegradable by STP B_MBR sludge, moderately biodegradable in STP A_AAO sludge under aerobic condition, while it was hardly biodegradable under anoxic condition and by STP B_CAS sludge, and no degradation under anaerobic condition. The biodegradable abilities of trimethoprim were much higher in anoxic and anaerobic conditions (k_{bio} >1) than those in aerobic conditions ($k_{bio} < 0.5$). Separately estimation and characterization on the contribution of AOB co-metabolism could help to better understand the mechanisms of biodegradation of each antibiotic in activated sludge, however, the estimation accuracy was higher for first-order kinetics due to less times of estimation. Therefore, the first-order biodegradation rate constants of target antibiotics would be applied for further estimation.

Thirdly, the integration of the primary and secondary treatment models was applied to estimate the fate of antibiotics in the STPs based on the estimated K_d and k_{bio} value, and the investigation on the fate of target antibiotics were also carried out in the two target STPs. All the target antibiotics can be detected in influent and primary effluent.

The concentrations of amoxicillin, ampicillin and cefazolin in secondary of effluent and effluent were below the limits of detection (LODs) in the two STPs. In STP A, the highest average concentration of target antibiotics detected in the effluent was for sulfamethoxazole (263 ng/L), and the concentrations of other compounds detected in the effluent were all lower than 100 ng/L. In STP B, the concentrations of all target compounds in MBR-effluent were lower than those in CAS-effluent. The estimated removal of antibiotics by wasting primary and excess sludge were acceptable, while for the biodegradation, the accuracy of predicted removal for the moderately and hardly biodegradable antibiotics were relatively low, which was mainly resulted from the lower estimated accuracy of k_{bio}. A fifteen-group classification on the estimated removal efficiency based on the K_d value and k_{bio} value of antibiotics was established, on the basis of this classification, a strategy for the prediction of antibiotics concentration in secondary effluent of target STPs was achieved and applied to obtain the PEC_{sec_eff} of antibiotics in target STPs. Generally, the PEC_{sec_eff} of antibiotics in STP A were lower than that in STP B due to the higher wastewater production per inhabitant per day and higher removal efficiency in STP A. The discharge of β-lactams was relatively low even the total consumption was high in Japan, which was resulted from the highly degradable character. For the antibiotics from other classes, even the removal in AAO system were higher than that in CAS system, the PEC_{sec_eff} from the target STP A were still high, which require further advanced treatment showed higher removal of antibiotics, e.g., ozonation. As the results in this study, MBR could also improve the biodegradation ability or shorten the half-lives of antibiotics, which could be considered as an alternative for the further STP upgrading.

Finally, the PEC_{sec_eff} of antibiotics from the two STPs were applied for the risk assessment by considering both environmental (ecotoxicity) and human health (AMR selection issue) perspectives. The $PNEC_{sw}$ (calculated by the ecotoxicity data) of target antibiotics shows a larger range from 19 ng/L to 100 μg/L than $PNEC_R$ (calculated by AMR selection data) (20 ng/L to 2683 ng/L). The $PNEC_{sw}$ value of ampicillin, azithromycin, cephalexin and sulfamethoxazole were lower than those of $PNEC_R$, while the $PNEC_R$ of other compounds were lower. The RQ values for STP A are lower than those of STP B due to the higher removal of antibiotics. For the PEC_{sec_eff} of STP A,

amoxicillin, ampicillin, piperacillin, clarithromycin, azithromycin and levofloxacin showed high risk with an average RQ value of 2.913, 1.167, 3.838, 22.425, 10.606 and 13.575, respectively. These above six antibiotics also showed high risk in STP B, with an average RQ value of 3.263, 1.071, 8.150, 36.300, 22.316 and 26.675, respectively. The average RQ value of ceftriaxone, meropenem and trimethoprim in STP A was below 1, while they showed high risk (average RQ = 3.375, 2.225 and 1.333, respectively) in STP B. For amoxicillin, ampicillin, piperacillin, ceftriaxone, meropenem, and trimethoprim, either secondary treatment enhancement strategy through advanced biological treatment or further treatment for secondary treatment strategy can help to fulfill the discharge target for these compounds; for clarithromycin, azithromycin and levofloxacin, the combinations of consumption reduction, secondary treatment enhancement and further treatment of secondary effluent should be considered.

Since the predicted removal of antibiotics in STPs highly depends on the estimation of biodegradation rate constant of antibiotics in the activated sludge, the long-time batch experiment (>72 h) on the study of biodegradation rate constant is needed to improve the accuracy of estimation in the future study. Even some antibiotics (e.g., β-lactam antibiotics) can be highly removed in STPs, however, the transformation by-products of these antibiotics, which might show ecotoxicity to microorganisms or select for the resistant bacteria, were not considered in this study. Therefore, it is necessary to further identify and quantify the transformation by-product of these antibiotics and test or estimate their ecotoxicity or MSCs. During the risk assessment, the single antibiotic data on the single species were applied, to make the proposed discharge limits closer to our protection goals, further studies are required to test the ecotoxicity or MSCs of mixtures of antibiotics on the natural microbial communities.

Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AAO: Anaerobic/anoxic/oxic

AMR: Antimicrobial resistance

AOB: Ammonia-oxidizing bacteria

AOPs: Advanced oxidation processes

ATU: Allylthiourea

BOD: Biochemical oxygen demand

CAS: Conventional activated sludge

COD: Chemical oxygen demand

DDD: Defined daily dose

DO: Dissolved oxygen

DOC: Dissolved organic carbon

ECs: Emergent contaminants

ESI: Electrospray ionization

HILIC: Hydrophilic interaction chromatography

HRT: Hydraulic retention time

LC-MS/MS: Liquid chromatography with tandem mass spectrometry

LODs: Limits of detection

MBR: Membrane bioreactor

MECs: Measured environmental concentrations

MHLW: Ministry of Health, Labor, and Welfare of Japan

MICs: Minimum inhibitory concentrations

MLSS: Mixed liquor suspended solid

MSCs: Minimum selective concentrations

PECs: Predicted environmental concentrations

PNEC: Predicted no effects concentration

PPCPs: Pharmaceutical and personal care products

QSAR: Quantitative Structure Activity Relationship

RMSE: Root mean square error

SPE: Solid-phase extraction

SRT: Sludge retention time

SS: Suspended solid

STP: Sewage treatment plant

Chapter I

Introduction

1.1 Research background

In the past two to three decades, the emerging micropollutants have been discharged to the environments due to their massive and continuous usage in our everyday life. Among them, antibiotics have raised worldwide concerns since the correlation between their ecotoxicity and development of antibiotic resistant and their consumption has been studied (Asai et al., 2005; Carvalho and Santos, 2016; Van De Sande-Bruinsma et al., 2008). For the further risk assessment, it is essential to monitor the concentrations of antibiotics released to the environment. Huge progress has been made on the development of antibiotics analysis in different environmental matrices (Rao et al., 2008; Petrovic et al., 2010; Gao et al., 2005). However, it is still difficult to establish a multi-target method to obtain acceptable recoveries for all antibiotics simultaneously due to the variations of chemical and physical properties of antibiotics from different classes and influence of environmental matrices. Moreover, monitoring of antibiotics is also restricted by the expensive cost and time consumption. Therefore, some researcher developed the prediction models based on the antibiotics consumption data and tried to estimate the release of antibiotics. The discharge of sewage treatment plants (STPs) is considered as the main route for human-used antibiotics entering into the aquatic environment (Michael et al., 2013; Oberoi et al., 2019). Therefore, the prediction models involved two main parts: I) to predict the antibiotics concentrations in the sewage influent; II) to predict the removal of antibiotics in the STPs.

After the consumption of antibiotics by humans, part of the parent compounds and their metabolites would discharge to the sewer system and diluted by the wastewater ((Le-Minh et al., 2010), which would finally enter into the STPs. Therefore, there are three relevant factors involved to predict the antibiotics concentrations in the sewage influent: the consumption of human-used antibiotics, excretion rate of each antibiotic from human bodies, wastewater produced volume per inhabitant in studied aera

(Carballa et al., 2008, Ort et al., 2009; Verlicchi et al., 2014). Among them, the consumption of human-used antibiotics and the excretion rate of each antibiotic are the two main factors affecting the accuracy of the prediction model since they cannot be characterized accurately by any available data sources (Azuma et al., 2015; Verlicchi et al., 2013). Hence, selection of proper data on the consumption and excretion rate of antibiotics become principal point in the prediction.

During the STPs, the antibiotics concentration in the stream is normally expressed as the sum of soluble concentration and its sorbed concentrations. Sorption and biodegradation are two main pathways for the removal of antibiotics in STPs (Hörsing et al., 2011; Wright, 2005). Therefore, the sorption distribution coefficient and biodegradation rate constant of antibiotics are two important parameters involved in the prediction models. Some previous researchers developed prediction models for individual unit in both water and sludge streams, such as physic-chemical separation units (Takács et al., 1991; Carballa et al., 2008), activated sludge units (Alvarino et al., 2014; Guo and Vanrolleghem, 2014), sludge thickening and dewatering units (Gernaey et al., 2014), anaerobic sludge digester units (Taboada-Santos et al., 2019) and so on. These prediction models are based on specific assumptions in each individual unit of STPs, while the mass balance is the basic concept in all models. In this study, we mainly focus on the discharge of antibiotics to aquatic environment, thus the prediction models on the water stream would be applied.

Even many prediction models have been developed, there is still no integrate of these models to predict the discharge of antibiotics based on the consumption data due to the variations of the concepts. Moreover, the investigation data on the occurrences of antibiotics should be obtained to evaluate the accuracy of the models.

It is necessary to characterize the risk of the estimated discharge of antibiotics from STP. Previous risk assessment work of antibiotic residues only considered the ecotoxicity, it is urgent to involve the potential risk on the selection and development of antibiotic resistance for antibiotic residues, based on which to propose the discharge limits of antibiotics.

1.2 Research objectives

Based on the above research background, detailed objectives of this research are as follows:

1. To predict the human-used antibiotics concentrations in the sewage influent based on Japanese annual shipping/prescription database and evaluate the accuracy of prediction models by the investigation data;
2. To compare the removal performances of antibiotics in the activated sludge from three different processes and estimate the sorption distribution coefficients and biodegradation rate constants of antibiotics;
3. To estimate the fate of antibiotics in the STPs through the integrate of plant-wide models and evaluate by the measured data;
4. To characterize the risk of predicted antibiotics concentration discharged from STPs and propose the strategies for the reduction of released antibiotics to fulfill the discharge goal.

1.3 Research structure

This dissertation consists of six chapters, the structure of this study is shown in Figure 1-1.

The research background, research objectives and research structure were described in Chapter I. In Chapter II, literature review was performed to summarize the knowledge of analytical methods of antibiotics, the consumption, occurrence and potential risk of human-used antibiotics in Japan, the removal of antibiotics in STPs, as well as the development of prediction models.

In Chapter III, the consumption of human-used antibiotics in Japan was calculated by both annual shipping volume and prescription volume, which were applied in the prediction model to obtain the PECs in the influent of two target STPs. Meanwhile, the antibiotics with high consumption and available analytical methods were investigated in the two target STPs to evaluate the accuracy of the prediction model.

In Chapter IV, the study on the removal performances of antibiotics in anaerobic/anoxic/oxic (AAO) sludge, conventional activated sludge (CAS) and

membrane bioreactor (MBR) sludge were carried out, in which the sorption distribution coefficient of antibiotics were obtained to estimate the constant of empirical predictive model, biodegradation rate constant of antibiotics were estimated, and the contribution of AOB co-metabolism was characterization as well.

In Chapter V, the prediction models in each individual unit of STPs were integrated to estimate the fate of antibiotics in the STPs, and the measured data were carried out to assess the accuracy of the plant-wide models. Finally, the discharge of antibiotics from STPs were evaluated.

In Chapter VI, risk assessment was carried out for estimated discharge of antibiotics, the discharge limit of individual antibiotic from the STPs and the possible strategies for the reduction of released antibiotics above the discharge limit were proposed.

Lastly, conclusions of this research and recommendations for further study were summarized in Chapter VII.

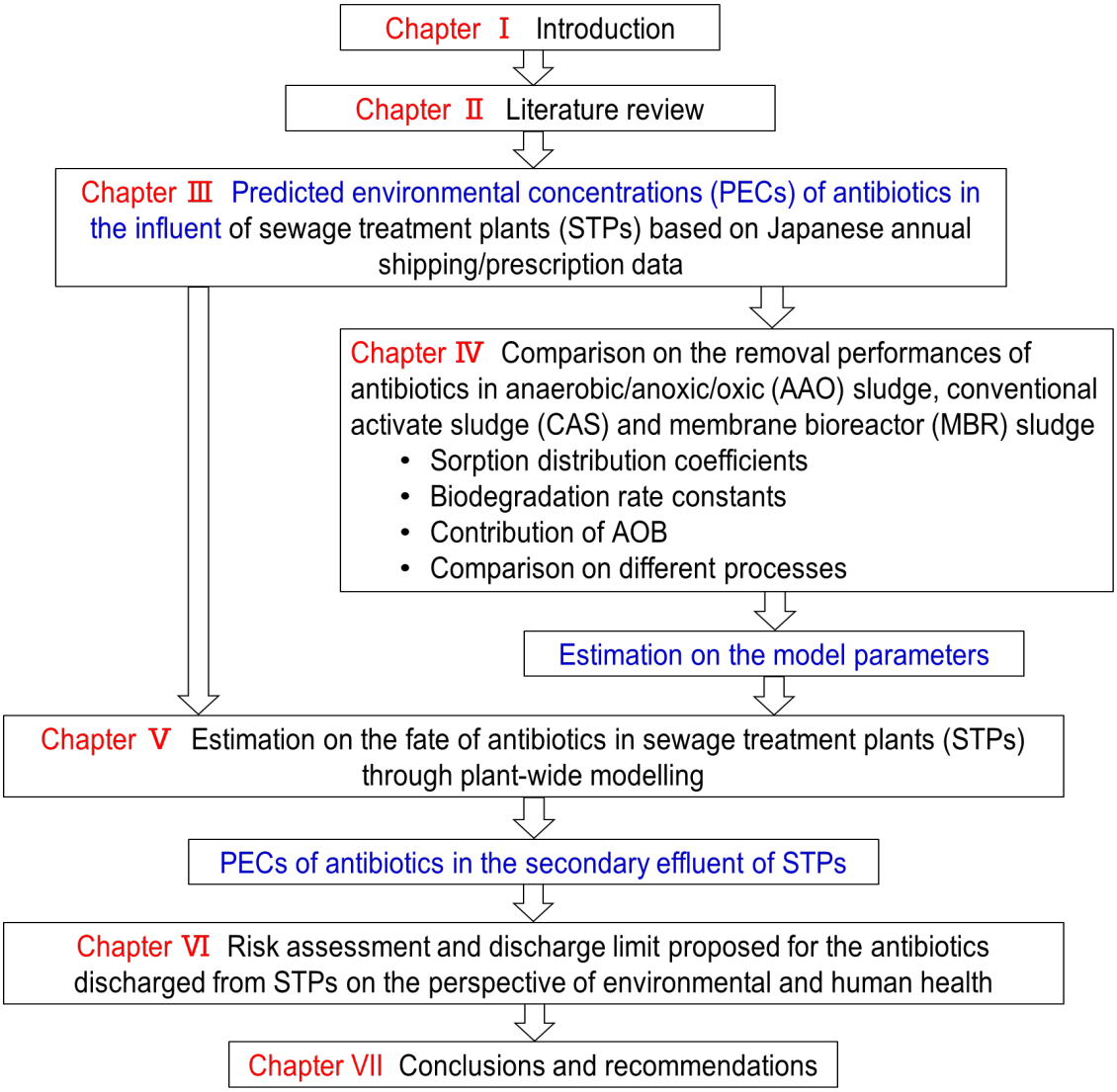


Figure 1-1 Schematic diagram of research structure

1.4 Reference

Alvarino, T., Suarez, S., Lema, J. M., Omil, F., 2014. Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. *Journal of Hazardous Materials*, *278*, pp.506-513.

Asai, T., Kojima, A., Harada, K., Ishihara, K., Takahashi, T. and Tamura, Y., 2005. Correlation between the usage volume of veterinary therapeutic antimicrobials and resistance in Escherichia coli isolated from the feces of food-producing animals in Japan. *Japanese journal of infectious diseases, 58*(6), pp.369.

Azuma, T., Nakada, N., Yamashita, N., Tanaka, H., 2015. Evaluation of concentrations of pharmaceuticals detected in sewage influents in Japan by using annual shipping and sales data. *Chemosphere*, *138*, pp.770-776.

Carballa, M., Fink, G., Omil, F., Lema, J. M., Ternes, T., 2008. Determination of the solid-water distribution coefficient (K_d) for pharmaceuticals, estrogens and musk fragrances in digested sludge. *Water Research*, *42*(1-2), pp.287-295.

Carballa, M., Omil, F., Lema, J. M., 2008. Comparison of predicted and measured concentrations of selected pharmaceuticals, fragrances and hormones in Spanish sewage. *Chemosphere*, *72*(8), pp.1118-1123.

Carvalho, I.T. and Santos, L., 2016. Antibiotics in the aquatic environments: a review of the European scenario. *Environment International, 94*, pp.736-75.

Gao, S., Zhang, Z.P., Karnes, H.T., 2005. Sensitivity enhancement in liquid chromatography/atmospheric pressure ionization mass spectrometry using derivatization and mobile phase additives. *Journal of Chromatography B*, *825*(2), pp.98-110.

Gernaey, K. V., Jeppsson, U., 2014. *Benchmarking of control strategies for wastewater treatment plants*. IWA publishing.

Guo, L., Vanrolleghem, P. A., 2014. Calibration and validation of an activated sludge model for greenhouse gases no. 1 (ASMG1): prediction of temperature-dependent N 2 O emission dynamics. *Bioprocess and Biosystems Engineering*, *37*(2), pp.151-163.

Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., la Cour Jansen, J., Andersen, H. R., 2011. Determination of sorption of seventy-five pharmaceuticals in sewage sludge. *Water research*, *45*(15), pp.4470-4482.

Le-Minh, N., Khan, S.J., Drewes, J.E. and Stuetz, R.M., 2010. Fate of antibiotics during municipal water recycling treatment processes. *Water research*, *44*(15), pp.4295-4323.

Michael, I., Rizzo, L., McArdell, C. S., Manaia, C. M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D., 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. *Water research*, *47*(3), pp.957-995.

Oberoi, A. S., Jia, Y., Zhang, H., Khanal, S. K., Lu, H., 2019. Insights into the fate and removal of antibiotics in engineered biological treatment systems: a critical review. *Environmental science & technology*, *53*(13), pp.7234-7264.

Ort, C., Hollender, J., Schaerer, M., Siegrist, H., 2009. Model-based evaluation of reduction strategies for micropollutants from wastewater treatment plants in complex river networks. *Environmental Science & Technology*, *43*(9), pp.3214-3220.

Petrovic, M., Farré, M., De Alda, M.L., Perez, S., Postigo, C., Köck, M., Radjenovic, J., Gros, M., Barcelo, D., 2010. Recent trends in the liquid chromatography–mass spectrometry analysis of organic contaminants in environmental samples. *Journal of Chromatography A*, *1217*(25), pp.4004-4017.

Rao, R.N., Venkateswarlu, N., Narsimha, R., 2008. Determination of antibiotics in aquatic environment by solid-phase extraction followed by liquid chromatography–electrospray ionization mass spectrometry. *Journal of Chromatography A*, *1187*(1-2), pp.151-164.

Taboada-Santos, A., Braz, G. H., Fernandez-Gonzalez, N., Carballa, M., Lema, J. M., 2019. Thermal hydrolysis of sewage sludge partially removes organic micropollutants but does not enhance their anaerobic biotransformation. *Science of The Total Environment*, *690*, pp.534-542.

Takács, I., Patry, G. G., Nolasco, D., 1991. A dynamic model of the clarification-thickening process. *Water Research*, *25*(10), pp.1263-1271.

Van De Sande-Bruinsma, N., Grundmann, H., Verloo, D., Tiemersma, E., Monen, J., Goossens, H., Ferech, M. and European Antimicrobial Resistance Surveillance System, 2008. Antimicrobial drug use and resistance in Europe. *Emerging infectious diseases, 14*(11), pp.1722.

Verlicchi, P., Al Aukidy, M., Jelic, A., Petrović, M., Barceló, D., 2014. Comparison of measured and predicted concentrations of selected pharmaceuticals in wastewater and surface water: a case study of a catchment area in the Po Valley (Italy). *Science of the Total Environment*, *470*, pp.844-854.

Verlicchi, P., Galletti, A., Petrovic, M., Barceló, D., Al Aukidy, M., Zambello, E., 2013. Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed—analysis of their respective contributions. *Science of the Total Environment*, *454*, pp.411-425.

Wright, G. D., 2005. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Advanced Drug Delivery Reviews*, *57*(10), pp.1451-1470.

Chapter II
Literature Review

2.1 Introduction

In recent years, as the development of chemical analysis technology, it became available to detect the emergent contaminants (ECs), such as pharmaceuticals and personal care products (PPCPs) in environment at low concentrations (Arpin-Pont et al., 2016; Kachhawaha et al., 2020; Primel et al., 2012). Due to their massive and continuous used in our everyday life, these chemicals were continuously discharged to the environment. Some of these chemicals are biologically active even at low concentrations and also can be accumulated in aquatic organisms (Tanoue et al., 2015). Then occurrence of these chemicals in aquatic environments has potential threats to human health and aquatic environment, which has been a topic with increasing concern. Numerous papers have been published on the analytical methods (Primel et al., 2012; Pérez-Lemus et al., 2019), occurrences (Kosma et al., 2010; Pompei et al., 2019; Yu and Chu, 2009) and ecotoxicity and risk of ECs (De García et al., 2014; Tamura et al., 2017) in the aquatic environment. However, the evaluation of ecotoxicity and risk were mostly focused on the individual chemicals, little is known in the real complicated aquatic environment. In the future, more appropriate and effective strategies should be established for the monitoring and risk assessment on ECs which have priorities based on consumption and ecotoxicity factors.

Among ECs, antibiotics raised worldwide concerns since the correlations between the emerging and development of antibiotic resistant and their consumption and occurrence in the environment had been studied (Asai et al., 2005; Bronzwaer et al., 2002; Van De Sande-Bruinsma et al., 2008). Antibiotics were used as human and veterinary medicines, which can effectively cure and reduce the pandemic of infectious diseases. After a short time of residence in human and animal's bodies, 30%-90% of the unchanged compounds and their active metabolites can be excreted through urine and feces (Carvalho and Santos, 2016; Massé et al., 2014). Since the conventional sewage

treatment can only partly remove these compounds (Michael et al., 2013; Oberoi et al., 2019), the discharge of sewage treatment plant (STP) effluent becomes the main source for human-used antibiotics and their active metabolites entering into the aquatic environments. Occurrences of antibiotics in the aquatic environment varied by many factors, such as consumption volume, excretion rate of individual compound, water usage volume, removal efficiency in STP and so on (Halling-Sørensen et al., 2001; Jones et al., 2002; Jones et al., 2005). To monitor all the antibiotics comprehensively in each area is a huge task which would cost lots of time, manpower and material resources. Besides that, there are still obstacles for detecting some compounds due to environmental matrix. Therefore, there is a need to develop a realistic strategy for predicting the environmental concentrations of antibiotics based on human consumptions. It has been reported that some researchers have been applied related predicting practical on sewage influents of some human pharmaceuticals (Azuma et al., 2015; Oosterhuis et al., 2013; Verlicchi and Zambello, 2016). In addition, some researchers also applied some methods to assess or predict the fate of pharmaceuticals during the STPs (Baalbaki et al., 2017; Taboada-Santos et al., 2020), which combined some empirical mechanistical models, such as hydraulic models, biodegradation kinetic models and so on. For the further risk assessment and establishment of proper guideline on the usage of antibiotics, it would be a promising way to combine proper models based on consumption data to estimate the discharge of human-used antibiotics.

2.2 Analytical methods

Due to the growing concern of antibiotics in different environmental matrices, huge progress has been made in the analytical methods development on antibiotic analysis. Reliable and accurate monitoring data on antibiotics are significant for the assessment of environmental risk and impacts on human health. Efficient, sensitive and fast analytical methods are required for monitoring antibiotics. Antibiotics are usually at low concentrations (ng/L) and also together with their interaction with complex aquatic environmental matrices, so the sample preparation is critical, which would extract and isolate the target compounds from the environmental matrices. The solid-phase extraction (SPE) was a preferred technique for sample purification and concentration

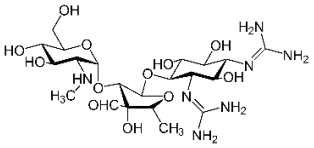
(Picó et al., 2007). Chelating agents such as Ethylenediaminetetraacetic acid (EDTA) and oxalic acid are usually added to the samples to decrease the binding of antibiotics to cations in the environmental matrices before SPE (Miao et al., 2004). Liquid Chromatography (LC) analysis is a choice for the determination of antibiotics after SPE. Nowadays, LC with tandem mass spectrometry (LC-MS/MS) and ultra-high-performance LC with tandem mass spectrometry (UHPLC-MS/MS) are new trends for the analysis of antibiotics due to higher sensitivity (Buchberger, 2007).

To make the analytical method more convenient and cost-effective, multi-residue method was developed to detect multi-target chemicals simultaneously. During this method, one of the biggest difficulties is to select proper sorbent and extracting conditions to achieve acceptable recovery rates of all analytes, which means the recovery rates of target analytes cannot reach their highest level. The most commonly used SPE cartridge for antibiotics is Oasis Hydrophilic-Lipophilic Balanced (HLB), which could pre-concentrate both polar and non-polar chemicals under the same condition (Rao et al., 2008; Tamtam et al., 2008). The elution process of cartridges is usually carried out by appropriate organic solvents (Caldas et al., 2011).

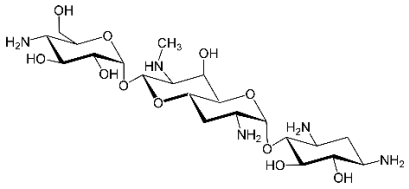
Techniques based on LC have been used for antibiotics detection in aquatic environment, the most recent methods depend on the use of MS/MS, which allows the limits of detections (LODs) in the range of ng/L and shortens the run time (Petrovic et al., 2010). Electrospray ionization (ESI) is the most important ionization technique on-line coupling with LC. Generally, reversed phase is the typical type for separation, and C18 (simply means that the molecules contain 18 carbon atoms) and C8 analytical columns are commonly used for the separation of antibiotics. Gradient elution programs with mixed solvent system (water/methanol or water/acetonitrile) were reported to improve the separation ability of multi-residues (Narumiya et al., 2013). Volatile compounds, such as formic acid, acetate acid and ammonium acetate, are often added to modify the mobile phase component for the improvement of ionization efficiency, which could improve the detection sensitivity of antibiotics (Gao et al., 2005). However, it is difficult to establish a multi-target method to detect all antibiotics simultaneously due to the variations of chemical and physical properties of antibiotics from different classes.

2.2.1 Aminoglycosides

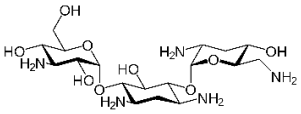
Aminoglycosides are broad-spectrum antibiotics characterized by two or more amino sugars linked by glycosidic bonds to an aminocyclitol component. Some representative aminoglycoside antibiotics and their structures are shown in Figure 2-1. Aminoglycoside antibiotics are water soluble and highly polar compounds, which result in difficulties to extract and preconcentrate from the aqueous samples. Kaufmann and Maden (2005) used the Oasis HLB cartridge tandem with the Sep-Pak cartridge (with Silica as sorbent), which could absorb the analytes of even weak hydrophobicity from aqueous solution, to extract 11 aminoglycosides and obtain acceptable recovery rates of all target compounds. Furthermore, aminoglycosides contain no chromophores or fluorophores which result in that UV or fluorescence detection cannot be used. In the commonly used reversed-phase LC analysis, these compounds show little retention because of their highly polar property, which is another challenge (Farouk et al., 2015). This problem has been overcome recent years by applying the hydrophilic interaction chromatography (HILIC) (Hemström and Irgum, 2006). There are several kinds of materials for HILIC column. Kumar et al. (2012) compared six different materials for the analysis of aminoglycosides and found that zwitter ionic (ZIC) materials showed the highest performance. Tao et al. (2012) applied HILIC column coupled with LC-MS/MS to simultaneously determine 15 aminoglycosides in animal derived foods and acceptable recovery rates (71-108%) were demonstrated. The detection of streptomycin in manure supernatant and rainfall run-off was carried out by a weak cation-exchange resin SPE cartridge (Oasis WCX) prior to the LC-MS/MS with HILIC column, 84% and 95% of recoveries in liquid hog manure supernatant and run-off water were obtained, respectively (Perum et al., 2006).



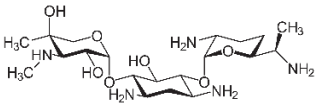
Streptomycin



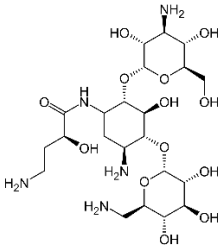
Apramycin



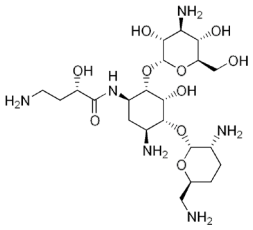
Tobramycin



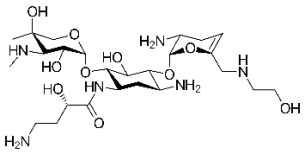
Gentamicin



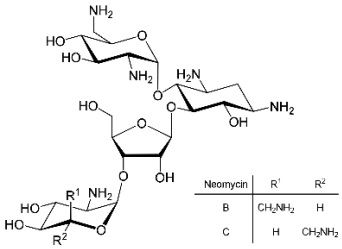
Amikacin



Arbekacin



Plazomicin



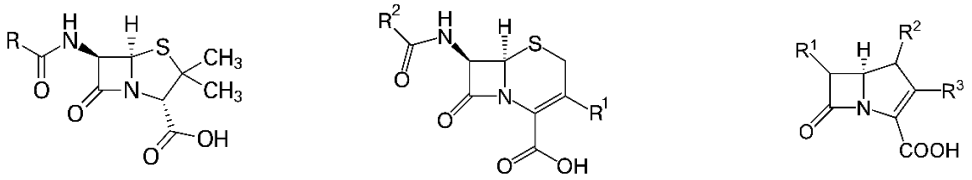
Neomycin B & C

Figure 2-1 Representative aminoglycoside antibiotics and their structures.

2.2.2 β-Lactams

β-Lactam antibiotics can be classified by their structural characteristics into several groups: penicillins, cephalosporins and carbapenems, but they all contain four-membered β-lactam (2-azetidinone) ring, their structures are shown in Figure 2-2. β-Lactam ring is susceptible to be degraded by a variety of reagents, chemical and enzymatic processes under various conditions. Penicillins can be easily hydrolyzed, and cephalosporins were demonstrated to be susceptible to chemical and enzymatic transformation (Deshpande et al., 2004). As a result, low occurrence levels of β-lactam antibiotics in the aquatic environment are expected, which require the analytical methods show very low LODs, especially during sample treatment procedures. Therefore, the multi-residues analytical methods are somehow limited for the determination of these antibiotics. Christian et al. (2003) plugged two different SPE

cartridges [200 mg of SDB-2 (styrene divinylbenzene as sorbent) and 200 mg of Oasis HLB] for multi-residues analysis of antibiotics to increase the extraction efficiency and thus improve the analytical sensitivity. Since β-lactam antibiotics are relatively small polar molecules, short retention is shown during the common reverse-phase LC-MS/MS analysis, the instrumental parameters should be optimized for multi-residues analysis to find a compromise. Schiesel et al. (2010) applied HILIC-ESI-MS/MS to detect β-lactam antibiotics and found that ZIC-HILIC column show the best compromise. However, Liu et al. (2011) compared HILIC Click β-CD column (150 mm × 2.1 mm i.d., 5 μm, 10 nm pore size, home made) and Atlantis HILIC Silica column (100 mm × 2.1 mm i.d., 5 μm, 10 nm pore size, Waters, USA) for the detection of seven cephalosporins and found that the separation performances of Click β-CD column were better than that of Atlantis HILIC Silica column. In their study, they also investigated the separation performances by reversed-phase high performance liquid chromatography (RP-HPLC) and obtained that the two separation modes had good orthogonality.



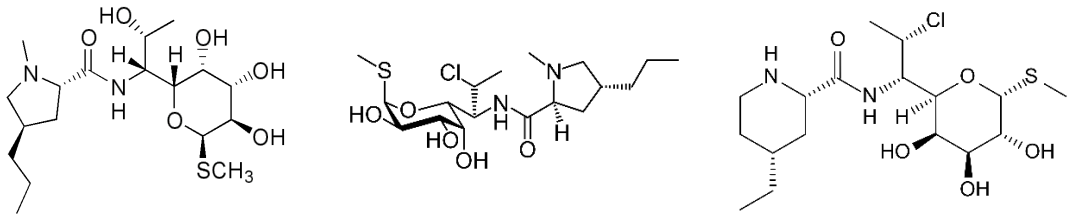
Penicillins Cephalosporins Carbapenems

Figure 2-2 β-lactam antibiotics structures.

2.2.3 Lincosamides

Lincosamides are a group of antibiotics consist of a pyrrolidine ring linked to a pyranose moiety via an amide bond, which are often grouped together with macrolides due to the similar mode of action. The most used lincosamides include lincomycin, clindamycin, and pirlimycin, and their structures are shown in Figure 2-3. The multi-residues analytical methods are usually successful for the detection of lincosamides. The most frequently detected lincosamides are lincomycin and clindamycin in the aquatic environment (Tran et al., 2016; Zuccato et al., 2010). Tran et al. (2016) compared three different SPE cartridges [Chromabond HR-X (polystyrene-divinylbenzen with super-crosslinked structure as sorbent), Chromabond SB (strong anion exchange resin with

specific surface area based on silica as sorbent) and Oasis HLB (hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene as sorbent)] under pH 3.0 and pH 7.0 conditions for the detection of antibiotics in different environmental matrices, high recoveries (80-105%) were shown for lincosamides by using Chromabond HR-X and tandem SPE cartridges (Chromabond SB+HR-X) under pH 3.0 condition. Watkinson et al. (2009) used Oasis HLB cartridge to extract the sample under pH 3.0 and high recoveries (>80%) also be obtained for detecting lincosamides.



Lincomycin Clindamycin Pirlimycin

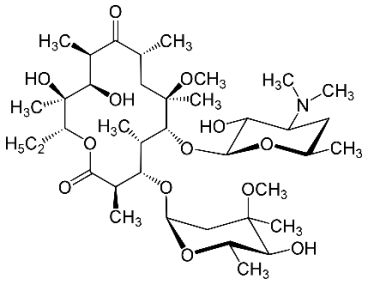
Figure 2-3 Representative lincosamides and their structures.

2.2.4 Macrolides

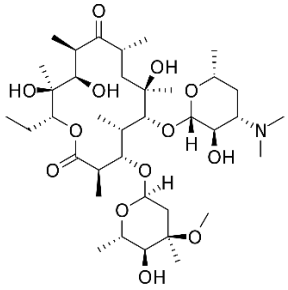
Macrolides are a group of basic and lipophilic molecules that characterized by a macrocyclic lactone ring containing 14, 15 or 16 atoms with sugars linked via glycosidic bonds. Based on the number of atoms, macrolides can be classified into three groups: clarithromycin, erythromycin and roxithromycin 14-membered macrolides, azithromycin is a 15-membered macrolide, whereas spiramycin and tylosin belong to 16-membered macrolides, and their structures are shown in Figure 2-4. Due to the poor UV absorbance of macrolides, the derivatization prior to LC with UV detector is needed for the analysis of these chemicals (Kanfer et al., 1998). Generally, the pK_a values of these chemicals are between 7.1 and 9.0, and some of macrolides are sensitive to pH and can be degraded under acid conditions (Horie et al., 1998). Therefore, the SPE extraction procedures become a limiting step for the multi-residue analytical methods. Erythromycin would be degraded to erythromycin-H_2O with loss of one molecule of water when the samples were adjusted to acid conditions during SPE extraction, as a result, many researchers measured erythromycin-H_2O alternative to erythromycin based on the assumption that erythromycin can be totally converted to erythromycin-H_2O after

adjusting pH to acid conditions (Yang and Carlson, 2006).

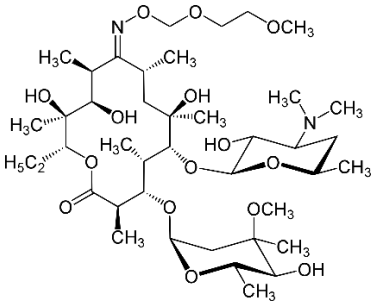
14-membered macrolides



Clarithromycin

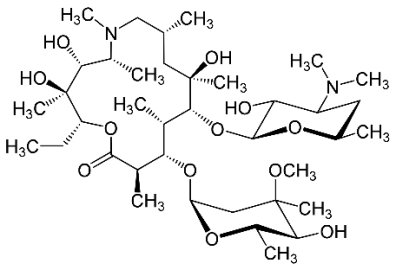


Erythromycin



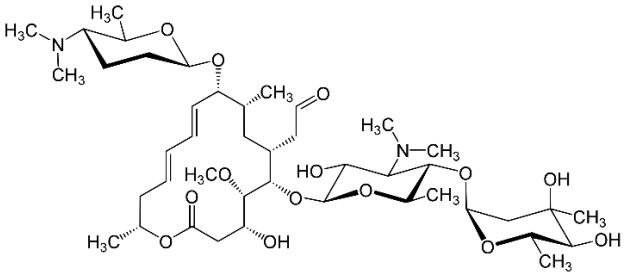
Roxithromycin

15-membered macrolides

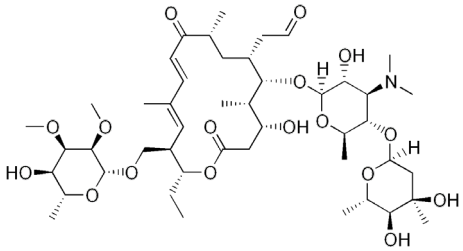


Azithromycin

16-membered macrolides



Spiramycin



Tylosin

Figure 2-4 Classification of macrolides and the structures of representative macrolides.

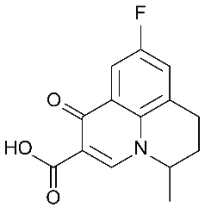
Several types of SPE cartridges have been used in the previous studies for the analysis of macrolides, like HLB, MCX, Strata-X, Strata-X-C, LiChrolute EN, LiChrolute RP-18, Isolut ENV+ and Strong anion exchange (SAX), and the HLB cartridges are the most frequently used one (Abuin et al., 2006; Gobel et al., 2004; Jacobsen et al., 2004; McArdell et al., 2003; McClure and Wong, 2007; Sacher et al., 2001; Stolker et al., 2004). The application of tandem cartridges (SAX+HLB) can reduce the matrices effects, wherein the SAX cartridges can bind negatively charged humic materials, and the HLB cartridges extract antibiotics (including macrolides), after

discharging the SAX cartridges and eluting antibiotics from the HLB cartridges, the solution contains less non-target organic compounds which would reduce the effects of environmental matrices (Jacobsen et al., 2004).

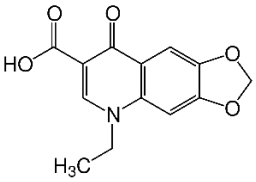
2.2.5 Quinolones

Quinolones are a class of broad-spectrum antibiotics, which can be classified into four generations based on their antimicrobial spectra (Figure 2-5). The pK_a values of these chemicals are between 5.9 and 6.3 for the carboxylic group, and range from 7.9-10.2 for the amino group. Generally, the multi-residues analytical methods are successful for the detection of quinolones. Jiménez-Lozano et al. (2004) carried out a comparative study of recovery rates of seven quinolones by different SPE cartridges (Zorbax C18, Bond Elut C18, Isolute ENV+, Oasis HLB and Oasis MAX) and found that the recoveries of all compounds were higher that 80% by using Oasis HLB and Isolute ENV+, and the recoveries were slightly better by using Oasis MAX, except for ciprofloxacin. While during the LC separation part, the highest peaks, well resolved and symmetric were obtained by using Oasis MAX cartridge, which means that lower LODs would be achieved. Due to the polarity character of quinolones, washing the SPE cartridges before elution by water or 5 % MeOH can decrease the matrix effects and suppression (Peng et al., 2008; Vieno et al., 2007). Dorival-García et al. (2013) recovered 98.5% to 103.9% of 13 quinolones from wastewater matrices (validating by matrices-match calibration) at pH 3.0 by applying Oasis HLB cartridges for SPE, and very low LODs (0.02-0.04 μg/L) of all target quinolones were achieved. Lombardo-Agüí et al. (2014) applied salting-out assisted liquid-liquid extraction (SALLE) coupled of UHPLC-MS/MS determine 19 quinolones in water samples, since further clean-up is not necessary after extraction, this method became faster and cheaper compared with SPE process, and relatively low LODs (0.01-0.09 μg/L) were obtained for all 19 quinolones.

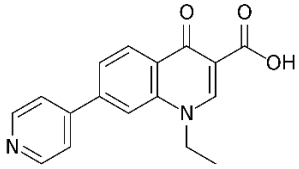
First-generation quinolones



Flumequine

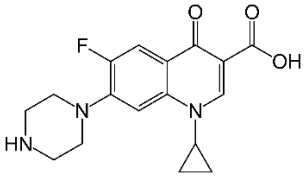


Oxolinic acid

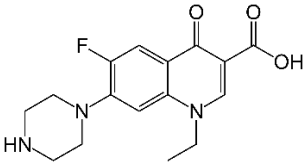


Rosoxacin

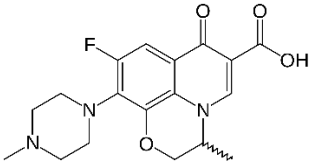
Second-generation quinolones



Ciprofloxacin

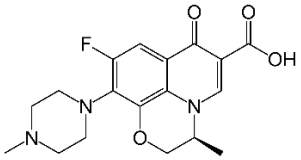


Norfloxacin

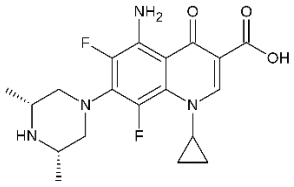


Ofloxacin

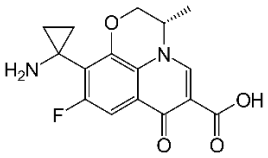
Third-generation quinolones



Levofloxacin

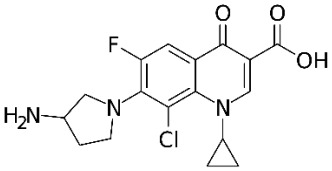


Sparfloxacin

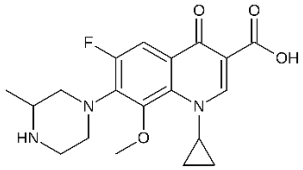


Pazufloxacin

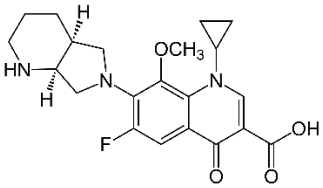
Fourth-generation quinolones



Clinafloxacin



Gatifloxacin



Moxifloxacin

Figure 2-5 Classification of quinolones and the structures of representative macrolides.

2.2.6 Sulfonamides and trimethoprim

Sulfonamides have been developed as antimicrobial agents since late 1930s, sulfamethoxazole combined with trimethoprim is currently most used, so these two compounds were usually simultaneously detected in environmental waters. The structures of trimethoprim and most commonly used sulfonamides are given in Figure 2-6. The general multi-residues analytical methods can provide reasonable performances for the determination of sulfonamides and trimethoprim. Pailler et al. (2009) compared the recoveries of 4 sulfonamides (sulfathiazole, sulfadimethoxine, sulfamethazine and sulfamethoxazole) by using Oasis HLB, Chromabond C-18EC, Chromabond EASY and Bond Elut PLEXA cartridges under pH 4.0 and natural

conditions with/without EDTA addition, the best recoveries (79-94%) of 4 sulfonamides were obtained by using HLB cartridges with EDTA at pH 4. Ye et al. (2007) detected 7 sulfonamides and trimethoprim in chlorinated drinking water by using single HLB cartridge at pH 3.0 with EDTA addition and LC-ESI-MS/MS analysis, acceptable recoveries (76-117%) and very low LODs (0.5-2.5 ng/L) were achieved. Sixteen sulfonamides and trimethoprim were detected in wastewater and river water matrices using HLB cartridges and UPLC-MS/MS, the overall recoveries in all matrices were 62-102%, and the LODs of this method for all compounds were 20-200 ng/L for influent, 16-120 ng/L for effluent and 8.0-60 ng/L for river water (Chang et al., 2008).

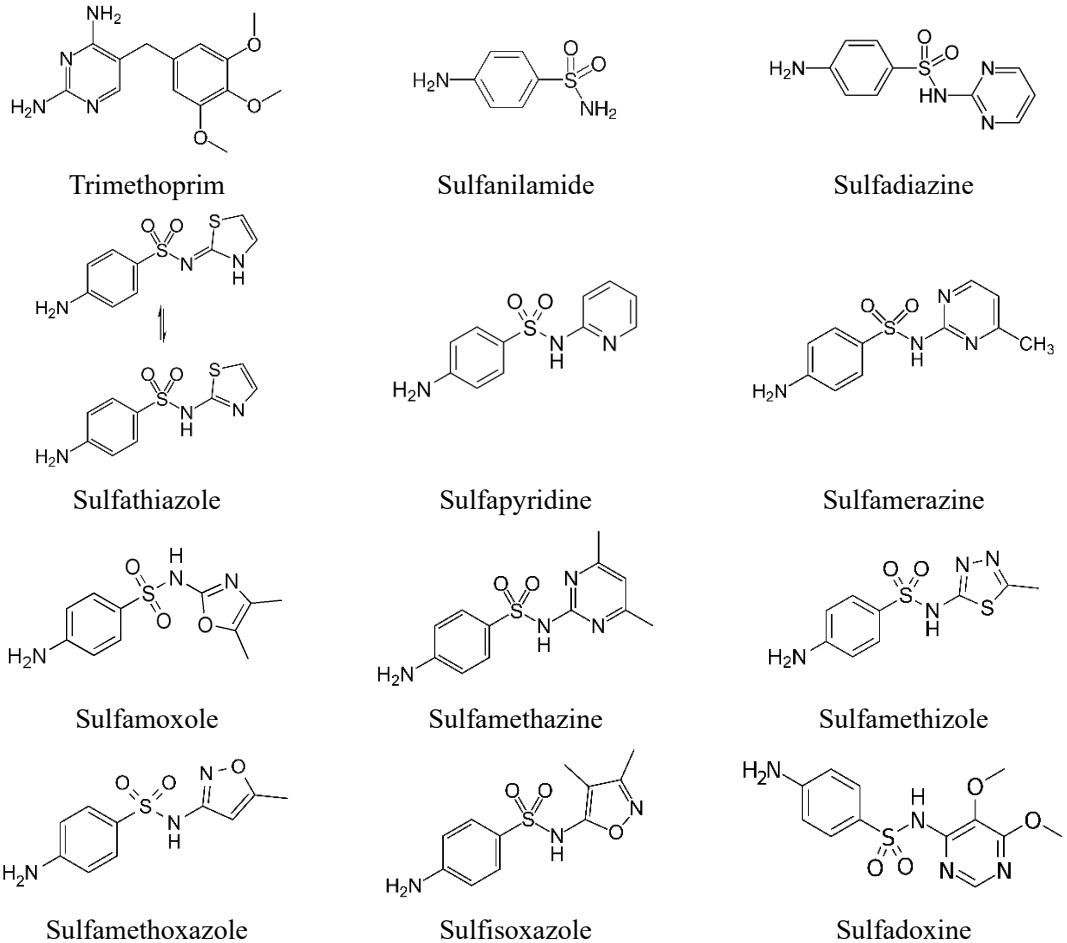
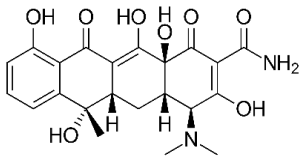


Figure 2-6 Structures of trimethoprim and representative sulfonamides.

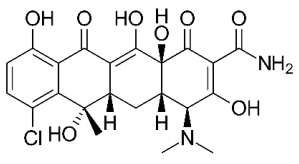
2.2.7 Tetracyclines

Tetracyclines are also a group of board-spectrum antibiotics which are not only

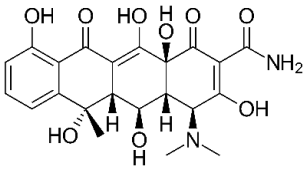
widely used in as veterinary medicines, but also as growth additives in feeds or water for livestock. Tetracycline molecules comprise an octahydronaphthacene ring skeleton with four fused rings (Figure 2-7). Their various functional groups such as carbonyl, hydroxyl, and amino sites can interact with aminopropyl and cyano in SPEs, as a result that strong anion exchange (SAX) cartridges are needed to remove the matrix interference before loading to Oasis HLB cartridge (Jacobsen and Halling-Sørensen, 2006). In addition, adding EDTA and adjusting pH below the lowest pK_a value before loading can help to improve the recoveries of tetracyclines from HLB cartridges (Jacobsen et al., 2004; Pailler et al., 2009). During LC analysis, tetracyclines are typically separated by the reversed phase columns (such as C_8 and C_{18} columns). However, these group of compounds can form chelate complexes with metal ion and absorb on the silanol group, which would cause extreme tailing of peaks (Anderson et al., 2005; Kahsay et al., 2014; Oka et al., 2000). One solution is to add chelating agents (e.g., oxalic acid, citric acid, EDTA and so on) to the mobile phase, which have been reported that optimize the peaks but cannot totally avoid this problem, except adding oxalic acid (Oka et al., 2000). The other solution is to use the amino-bonded silica HILIC columns to prevent these non-selective reactions (Kahsay et al., 2014). Some successful application of HILIC separation for tetracyclines have been reported, which were also further applied for the analysis of environmental samples (Li et al., 2011; Valette et al., 2004).



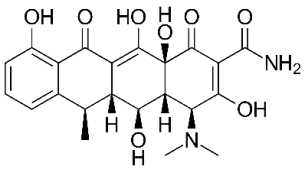
Tetracycline



Chlortetracycline



Oxytetracycline



Doxycycline

Figure 2-7 Representative tetracyclines and their structures.

2.3 Antibiotics: The Japanese scenario

2.3.1 Consumption

Antibiotics has been used as human and veterinary drugs across Japan. In human drugs, antibiotics were mainly used to control and treat infectious diseases. While in veterinary drugs, antibiotics were not only for the therapeutic uses in food producing animals, aquatic animals and companion animals, but also for antibiotic feed additives and agrochemicals. A survey commisioned by the Ministry of Health, Labour, and Welfare of Japan (MHLW) reported that between 2013 and 2017, the total volume of antibiotic consumption increased by 5.4%, from 1723.9 t to 1816.2 t. Human use represent 32%-34% and did not vary much between 2013 and 2017.

The MHLW also provides information about the specific human antibiotic consumption in Japan, expressed as defined daily dose (DDD) per 1000 inhabitants per day (DID). Table 2-1 shows the sales amount of human antibiotics in Japan between 2013 and 2018. The specific human antibiotic consumption were comparable between 2013 and 2016, while it decreased 5.4 % in 2017, and 8.8% in 2018 compared to 2016. Antibiotic use is mainly responsible for the antimicrobial resistance (AMR), the Japanese government developed a national action plan on AMR in 2016, to control the antibiotic consumption is one of the goals, which may cause the decrease of human antibiotic consumption. In 2018, the specific human antibiotic consumption is 13.31 DID, which is comparable to the developed EU countries (Figure 2-8). Penicillins were less used in Japan compared to the EU countries, while cephalosporins, macrolides and quinolones (broad-spectrum oral antibiotics) were frequently used in Japan.

Table 2-1 Trends in human antibiotic use in Japan based on the volume of sales (Ministry of Health, Labor, Welfare of Japan, 2019)

Antibiotic class name	DDD per 1000 inhabitants per day (DID)					
	2013	2014	2015	2016	2017	2018
Tetracyclines	0.78	0.77	0.79	0.82	0.83	0.9
Amphenicols	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
β -Lactam antibacterial, penicillins	1.26	1.30	1.43	1.44	1.44	1.56
1st generation cephalosporins	0.20	0.20	0.21	0.21	0.22	0.23
2nd generation cephalosporins	0.41	0.40	0.39	0.39	0.38	0.37
3rd generation cephalosporins	3.71	3.60	3.67	3.54	3.31	3.07
4th generation cephalosporins	0.06	0.05	0.05	0.05	0.05	0.04
Carbapenems	0.12	0.13	0.13	0.13	0.12	0.12
Other cephalosporins and penem	0.14	0.14	0.13	0.12	0.12	0.11
Sulfonamides and trimethoprim	0.25	0.27	0.29	0.31	0.33	0.36
Macrolides	4.83	4.50	4.59	4.56	4.18	3.96
Lincosamides	0.03	0.03	0.04	0.03	0.04	0.04
Streptogramins	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Aminoglycosides	0.05	0.05	0.05	0.04	0.04	0.04
Quinolones	2.87	2.87	2.75	2.79	2.61	2.38
Glycopeptides	0.03	0.03	0.03	0.03	0.03	0.03
Others	0.12	0.12	0.12	0.12	0.11	0.10
Total	14.89	14.46	14.67	14.60	13.81	13.31

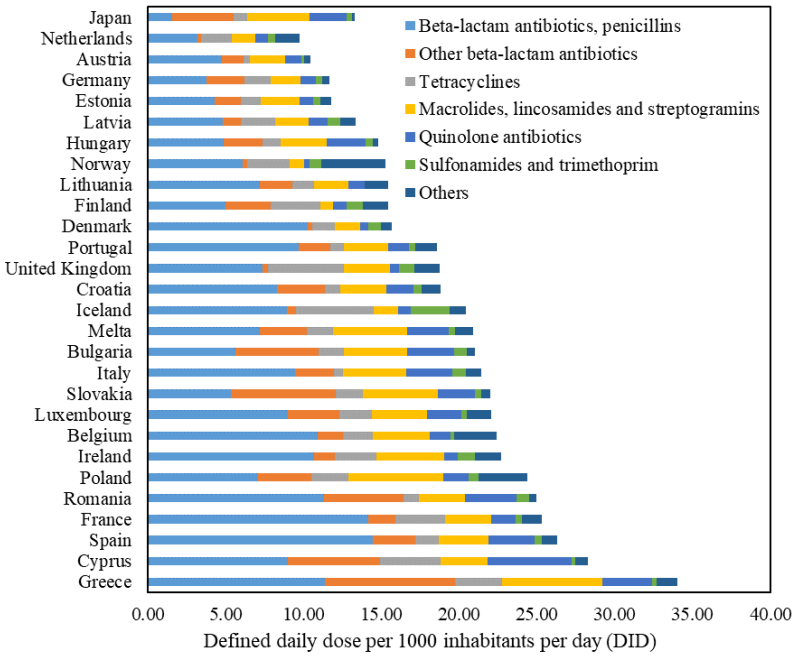


Figure 2-8 Comparison of human antibiotic use of EU countries (ECDC, 2019) and Japan in 2018.

2.3.2 Occurrence in surface waters

The aquatic ecosystem can supply food for humans and the surface waters are main sources of drinking water. Therefore, the contamination of aquatic environment can result in the direct threats to human health. Murata et al. (2011) did a national wide survey on 7 sulfamides, trimethoprim and 4 macrolides in 37 Japansese rivers and found that the sum of total selected antibiotics concentration ranged from undetectable to 626 ng/L, which were higher in urban rivers than that in rural rivers. Additionally, macrolides is dominant over sulfamides in urban rivers, and the concentration of clarithromycin was higher than 100 ng/L in some urban rivers, which is coinsident with the consumption data. Moreover, their observations also show that the selected antibiotics in Japanese rivers are mainly derived from the urban STPs. Compared the concentrations of antibiotics in the European rivers, macrolides antibiotics concentrations varied from 1 to 500 ng/L (Carvalho and Santos, 2016), higher concentrations were detected in French and Spanish rivers, which were ranging from 1 to 17 μg/L (Felizzola and Chiron, 2009; Valcárcel et al., 2011); the concentrations of sulfamides antibiotics were comparable in European river water, which were also generally detected below 100 ng/L (Chitescu et al., 2015; García-Galán et al., 2011; Gros et al., 2013). Adachi et al. (2103) collected water samples and detected fluoquinolones from 30 sampling sites in aquatic environment in Osaka, Japan and found that their concentrations were ranging from 0.1 to 570 ng/L.While in European river water, the concentrations of fluoquinolones were higher. For example, ciprofloxacin was detectd at maximum concentration of 9.7 μg/L in Arc River in France (Felizzola and Chiron, 2009) and 2.7 μg/L in Gościcina and Reda Rivers in Poland (Wagil et al., 2014); Ofloxacin was detected at concentration of 2 μg/L in Spanish (Roldán et al., 2010). Simazaki et al. (2015) investigated 6 rivers and ground water across Japan to detect 3 tetracyclines, it shows that the maximum concentrations of detected antibiotics in the rivers and ground water were all lower than 50 ng/L, which normally did not exceed tens ng/L in European rivers (Dinh et al., 2011; López-Serna et al., 2010). Few study investigated the occurrence of β-lactam antibiotics in the surface waters in Japan. On one hand, β-lactam antibiotics all contain the β-lactam nucleus, which is susceptible to cleavage by various reagents, biotic and abiotic processes, as a

results, it was expected that β-lactam antibiotics show relatively low concentration level in aquatic environment (Deshpande et al., 2004; Mitchell et al., 2014). On the other hand, there are still determining limitation by common multi-compounds methods in the aquatic environmental matrices. However, several researchers detected these antibiotics in European river waters and reported that the detected ranges of penicillins were 3.57-522 ng/L, cephalosporins were below 10 ng/L (Alygizakis et al., 2016; Carvalho and Santos, 2016).

2.3.3 Ecotoxicity and resistance of antibiotics in aquatic environment

Antibiotics, not only like other pharmaceuticals, show the potential risk to the ecosystem, but also expose the potential risk to the antibiotic resistance which might further impact human health (Grenni et al., 2018; Knapp et al., 2008). Current risk assessment work mainly focuses on their ecotoxicity, and the environmental risk assessment (ERA) proposed by EMEA (2018) aims to establish the safe concentrations for the protection of ecosystem by the calculations of ecotoxicity [e.g., predicted no effect concentration (PNEC)] of micropollutants, including antibiotics. However, the potentially enrichment concentration of AMR might be lower than the concentration of ERA ecotoxicity inhibition tests (Le Page et al., 2017). It is urgent to involve the AMR issue in the risk assessment framework for antibiotic residues.

2.3.3.1 Antibiotics ecotoxicity

Antibiotics are used to treat microbial infections which show antimicrobial activities, when these compounds release into the aquatic environment, they will not only influence the target organisms but also affect the non-target organisms (Grenni et al., 2018). Previous studies have shown that antibiotics can drive changes of the natural bacterial community structures and reduce the microbial diversity (Allen et al., 2010), and the effects can also be found in non-target organisms which have important ecological functions (Pallecchi et al., 2008). Furthermore, antibiotics can affect the growth and enzymes activities of organisms which further influence the ecological functions such as nutrient transformation and biomass production and lead to the loss of

functional stability (Martinez, 2009; Pallecchi et al., 2008; Pauwels and Verstraete, 2006). However, the potential of these ecotoxicological effects is difficult to predict and assess, especially in the complicated aquatic environmental matrices. Many researchers studied the acute and chronic ecotoxicity of antibiotics by conventional ecotoxicity assays on different organisms, such as algae, bacteria, fish and so on. Some of these studies on the ecotoxicological effects of antibiotics are summarized on Table 2-2.

Holten Lützhøft et al. (1999) compared the sensitivity of cyanobacteria, green algae and cryptophyte to different classes of antibiotics, the growth inhibiting effects of eight antibiotics were investigated. The results show that *Microcystis aeruginosa* (freshwater cyanobacteria) is more sensitive than *Selenastrum capricornutum* (fresh water green algae) and *Rhodomonas salina* (marine cryptophyte), the toxicity orders vary by different species. Amoxicillin and sulfonamides antibiotics were more toxic to cyanobacteria, oxytetracycline was more toxic to green algae and cryptophyte. Halling-Sørensen (2000) also compared the sensitivity of cyanobacteria and green algae to other antibiotics and got similar results. Eguchi et al. (2004) found that erythromycin (macrolides) showed the strongest toxicity to *Selenastrum capricornutum* (green algae), ampicillin and cefazolin (β-lactams) did not inhibit the growth. Moreover, sulfonamides antibiotics and trimethoprim showed some inhibitory activities, while the inhibiting effects of combined sulfonamides and trimethoprim (commonly used as combined drugs) were significantly improved. Yang et al. (2008) reported that the toxicity values (IC_{50}, the median inhibitory concentration) order on *Pseudokirchneriella subcapitata* (freshwater green algae): roxithromycin (0.056 mg/L) > clarithromycin (0.062 mg/L) > tylosin (0.20 mg/L) > tetracycline (2.25 mg/L) > chlortetracycline (3.49 mg/L) > norfloxacin (5.64 mg/L) > sulfamethoxazole (7.50 mg/L) > ciprofloxacin (20.22 mg/L) > sulfamethazine (31.26 mg/L) > trimethoprim (137.78 mg/L).

Table 2-2 Summary of aquatic toxicity of antibiotics

Antibiotic classes / Antibiotic compounds	Test organisms	Toxicity (EC_{50}, mg/L)	Bioassay	Exposure time	Reference
Aminoglycosides					
Streptomycin	*Microcystis aeruginosa*	0.007	Cyanobacteria test	7 days	(Halling-Sørensen, 2000)
	Selenastrum capricornutum	0.133	Green algae test	7 days	(Halling-Sørensen, 2000)
	Selenastrum capricornutum	0.107	Green algae test	3 days	(Eguchi et al., 2004)
β-Lactams					
Amoxicillin	*Microcystis aeruginosa*	0.0037	Cyanobacteria test	7 days	(Lützhøft et al., 1999)
	Rhodomonas salina	3108	Green algae test	7 days	(Lützhøft et al., 1999)
	Selenastrum capricornutum	>250	Green algae test	7 days	(Lützhøft et al., 1999)
Ampicillin	*Selenastrum capricornutum*	>1000	Green algae test	3 days	(Eguchi et al., 2004)
	Chlorella vulgaris	>1000	Green algae test	3 days	(Eguchi et al., 2004)
Benzylpenicillin (Penicillin G)	*Microcystis aeruginosa*	0.006	Cyanobacteria test	7 days	(Halling-Sørensen, 2000)
	Selenastrum capricornutum	>100	Green algae test	7 days	(Halling-Sørensen, 2000)
Cefazolin	*Selenastrum capricornutum*	>1000	Green algae test	3 days	(Eguchi et al., 2004)
Macrolides					
Clarithromycin	*Pseudokirchneriella subcapitata*	0.046	Green algae test	3 days	(Yang et al., 2009)
Erythromycin	*Selenastrum capricornutum*	0.0366	Green algae test	3 days	(Eguchi et al., 2004)
	Chlorella vulgaris	33.8	Green algae test	3 days	(Eguchi et al., 2004)
Roxithromycin	*Pseudokirchneriella subcapitata*	0.047	Green algae test	3 days	(Yang et al., 2009)
Tylosin	*Microcystis aeruginosa*	0.034	Cyanobacteria test	7 days	(Halling-Sørensen, 2000)
	Selenastrum capricornutum	1.38	Green algae test	7 days	(Halling-Sørensen, 2000)
	Pseudokirchneriella subcapitata	0.21	Green algae test	3 days	(Yang et al., 2009)
	Selenastrum capricornutum	0.44	Green algae test	3 days	(Eguchi et al., 2004)
Quinolones					
Ciprofloxacin	*Pseudokirchneriella subcapitata*	6.7	Green algae test	3 days	(Yang et al., 2009)
Norfloxacin	*Pseudokirchneriella subcapitata*	1.8	Green algae test	3 days	(Yang et al., 2009)
	Selenastrum capricornutum	16.6	Green algae test	3 days	(Eguchi et al., 2004)
	Chlorella vulgaris	10.4	Green algae test	3 days	(Eguchi et al., 2004)
Oxolinic acid	*Microcystis aeruginosa*	0.180	Cyanobacteria test	7 days	(Lützhøft et al., 1999)
	Rhodomonas salina	10	Green algae test	7 days	(Lützhøft et al., 1999)
	Selenastrum capricornutum	16	Green algae test	7 days	(Lützhøft et al., 1999)
Sulfamides and trimethoprim					
Sulfadiazine	*Microcystis aeruginosa*	0.135	Cyanobacteria test	7 days	(Lützhøft et al., 1999)
	Rhodomonas salina	403	Green algae test	7 days	(Lützhøft et al., 1999)
	Selenastrum capricornutum	16	Green algae test	7 days	(Lützhøft et al., 1999)
	Selenastrum capricornutum	2.19	Green algae test	3 days	(Eguchi et al., 2004)
Sulfamethazine	*Pseudokirchneriella subcapitata*	8.7	Green algae test	3 days	(Yang et al., 2009)
Sulfadimethoxine	*Selenastrum capricornutum*	2.3	Green algae test	3 days	(Eguchi et al., 2004)
	Chlorella vulgaris	11.2	Green algae test	3 days	(Eguchi et al., 2004)
Sulfamethoxazole	*Pseudokirchneriella subcapitata*	1.9	Green algae test	3 days	(Yang et al., 2009)
	Selenastrum capricornutum	1.53	Green algae test	3 days	(Eguchi et al., 2004)
Trimethoprim	*Microcystis aeruginosa*	112	Cyanobacteria test	7 days	(Lützhøft et al., 1999)
	Rhodomonas salina	16	Green algae test	7 days	(Lützhøft et al., 1999)
	Selenastrum capricornutum	130	Green algae test	7 days	(Lützhøft et al., 1999)
	Pseudokirchneriella subcapitata	40	Green algae test	3 days	(Yang et al., 2009)
	Selenastrum capricornutum	80.3	Green algae test	3 days	(Eguchi et al., 2004)
Tetracyclines					
Chlortetracycline	*Microcystis aeruginosa*	0.05	Cyanobacteria test	7 days	(Halling-Sørensen, 2000)
	Selenastrum capricornutum	3.1	Green algae test	7 days	(Halling-Sørensen, 2000)
	Pseudokirchneriella subcapitata	1.8	Green algae test	3 days	(Yang et al., 2009)
Oxytetracycline	*Microcystis aeruginosa*	0.207	Cyanobacteria test	7 days	(Lützhøft et al., 1999)
	Rhodomonas salina	1.6	Green algae test	7 days	(Lützhøft et al., 1999)
	Selenastrum capricornutum	4.5	Green algae test	7 days	(Lützhøft et al., 1999)
	Selenastrum capricornutum	0.342	Green algae test	3 days	(Eguchi et al., 2004)
	Chlorella vulgaris	7.05	Green algae test	3 days	(Eguchi et al., 2004)
Tetracycline	*Microcystis aeruginosa*	0.09	Cyanobacteria test	7 days	(Halling-Sørensen, 2000)
	Selenastrum capricornutum	2.2	Green algae test	7 days	(Halling-Sørensen, 2000)
	Pseudokirchneriella subcapitata	1.0	Green algae test	3 days	(Yang et al., 2009)

In addition, some researchers also tried to use Quantitative Structure Activity

Relationship (QSAR) models, which firstly summarized the relationship between chemical structures and biological activity in a data-set of chemicals and then predict the activity of new chemicals, to perform ecotoxicological (quantitative) structure activity relationship (ECOSAR) scanning of antibiotics (De García et al., 2014; Sanderson et al., 2003; Sanderson et al., 2004). Even they used the same tool (ECOSAR software) and method to predict the toxicity values of antibiotics on algae, crustacean and fish, their results were inconsistent, some compounds even present different orders of the values. Therefore, further improvements are needed on the methodology to get more reliable results.

Generally, most studies evaluated the ecotoxicity values of individual antibiotics or common combined drugs. However, antibiotics and their active transformation by-products (BPs) exist as a mixture in the complex aquatic environment matrices. For the ecological risk assessment, further studies are need on the ecotoxicity of antibiotics and their active BPs to provide available methodology for the real scenario assessment.

2.3.3.2 Antibiotic resistance

Generally, antibiotics were produced from fungal and bacteria, while the synthesis of artificial antibiotics are demanded by time since last few decades due to the resistance acquired by bacteria. Long-term exposure of antibiotics below the minimum inhibitory concentrations (MIC) can select for the antibiotic resistant bacteria (Knapp et al., 2008). As a result, overuse, underuse and misuse of antibiotics continuously bring new cases of bacterial resistances. In recent years, antibiotic resistance become a global phenomenon which induce the public health crisis, and the World Health Organization (WHO) has created a 5-objective global action plan to address this issue and coordinate numerous international sectors (Morehead and Scarbrough, 2018). In response to this, Japanese government published “National Action Plan on Antimicrobial Resistance (AMR) (2016-2020)” in April 2016, clearly indicating that the overall one health surveillance on antibiotic resistant bacteria isolated from humans, animals, food and environment has been performed, which could help to evaluate the impact of the action plan on AMR and plan the future national policies. Nippon AMR One Health Reports (NAORs) by years present the results of this surveillance, including the current status and trends of national consumption of antibiotics and antibiotic resistant bacteria in the

areas of human health, animals, agriculture, food and the environment. Table 2-3 shows the proportion of representative antibiotic-resistant bacteria isolated from human in 2018. It is worth noting that the proportion of *Escherichia coli* resistant to penicillins (e.g., ampicillin and piperacillin), first- and third-generation cephalosporins and fluoroquinolones (e.g., levofloxacin) were high, and according to the report that the proportion to third-generation cephalosporins and fluoroquinolones increased by years, calling for the action to address this issue.

Table 2-3a. The proportion (%) of representative antibiotic resistant bacteria (gram-negative) isolating from human in 2018 (Ministry of Health, Labour, Welfare of Japan, 2019)

Antibiotic Type	**BP***	**Gram-negative bacteria**					
		Escherichia coli	***Klebsiella pneumoniae***	***Enterobacter cloacae***	***Klebsiella aerogenes***	***Pseudomonas aeruginosa***	***Acintobacter spp.***
Ampicillin	32	52.2 (325,553)	79.4 (158,654)	81.2 (64,820)	80.3 (32,746)	-	-
Piperacillin	128	46.0 (342,066)	22.9 (165,430)	21.2 (66,020)	17.4 (33,048)	10.0 (206,858)	10.3 (27,905)
Piperacillin/ tazobactam	4/ 128	1.7 (263,131)	2.6 (127,778)	9.8 (52,186)	6.9 (26,272)	8.1 (172,748)	9.4 (12,171)
Cefazolin	8	38.7 (347,491)	14.3 (166,906)	98.3 (68,017)	95.0 (33,996)	-	-
Cefmetazole	64	0.9 (348,832)	1.6 (168,787)	88.0 (68,013)	89.1 (34,051)	-	-
Cefotaxime	4	27.5 (251,068)	9.4 (122,459)	32.9 (51,470)	33.4 (25,493)	-	-
Ceftazidime	16	12.4 (352,819)	5.7 (169,097)	26.3 (68,737)	27.8 (34,142)	8.4 (203,554)	7.6 (28,077)
Cefepime	32	16.7 (321,745)	5.8 (156,485)	3.9 (64,337)	1.4 (32,216)	6.0 (194,385)	6.8 (26,616)
Aztreonam	16	19.3 (273,064)	6.7 (133,009)	24.9 (55,988)	19.2 (28,281)	13.1 (162,365)	-
Imipenem	4	0.1 (321,043)	0.3 (154,879)	1.1 (63,611)	2.6 (31,288)	16.2 (188,778)	2.0 (16,995)
Meropenem	4	0.1 (365,600)	0.5 (175,408)	1.1 (71,119)	0.8 (35,448)	10.9 (209,149)	1.5 (29,024)
Amikacin	64	0.1 (362,591)	0.1 (174,259)	0.1 (70,659)	0.1 (35,214)	0.9 (209,413)	2.0 (28,437)
Levofloxacin	8	40.9 (360,329)	3.1 (172,010)	3.2 (69,392)	0.9 (34,383)	10.2 (199,760)	7.0 (28,209)

*BP: break point, μg/mL;

Figures in parentheses indicate the number of bacterial strains that were tested for antimicrobial susceptibility;

-: Not under surveillance

Table 2-3b. The proportion (%) of representative antibiotic resistant bacteria (gram-positive) isolating from human in 2018 (Ministry of Health, Labour, Welfare of Japan, 2019)

Antibiotic Type	BP*	Gram-positive bacteria				
		Staphylococcus aureus	**Methicillin-susceptible *Staphylococcus aureus* (MSSA)**	**Methicillin-resistant *Staphylococcus aureus* (MRSA)**	***Enterococcus faecalis***	***Enterococcus faecium***
Penicillin G	0.25	75.4 (287,805)	52.9 (135,360)	-	0.9 (104,023)	87.5 (42,178)
Ampicillin	16	-	-	-	0.2 (119,014)	87.6 (49,207)
Cefazolin	32	20.7 (360,772)	<0.05 (164,909)	-	-	-
Amoxicillin/ clavulanic acid	8/4	-	0.1 (26,376)	-	-	-
Imipenem	16	-	<0.05 (149,454)	-	-	-
Erythromycin	8	51.7 (325,918)	23.1 (150,809)	81.7 (159,215)	52.7 (102,496)	83.0 (43,555)
Clindamycin	4	22.0 (340,953)	2.7 (155,141)	41.7 (169,049)	-	-
Minocycline	16	12.2 (377,507)	0.6 (169,953)	23.7 (189,813)	50.9 (128,160)	38.3 (54,540)
Levofloxacin	4	50.4 (358,941)	13.8 (161,691)	86.8 (179,731)	10.4 (122,551)	86.7 (51,003)
Vancomycin	16	0.0 (374,982)	-	0.0 (189,853)	<0.05 (129,545)	0.9 (54,279)
Teicoplanin	32	<0.05 (336,502)	-	<0.05 (169,651)	<0.05 (115,397)	0.6 (48,991)
Linezolid	8	<0.05 (286,366)	-	<0.05 (144,332)	-	0.1 (41.596)
Daptomycin	2	0.3 (72,401)	-	0.5 (35,618)	-	-

*BP: break point, μg/mL;

Figures in parentheses indicate the number of bacterial strains that were tested for antimicrobial susceptibility;

-: Not under surveillance

2.4 STPs: Hotspots for the release of antibiotics

After the consumption of antibiotics, only a small fraction of both human and veterinary antibiotics can be degraded and absorbed in the body, it was reported that 30-90% of the consumed antibiotics were excreted through urine and feces (Carvalho and Santos, 2016; Massé et al., 2014). These antibiotics and their metabolites are finally discharged into the STPs. Since the STPs are not specifically designed for the removal of antibiotics, they cannot be removed completely in the STPs and their removal performances varies a lot depending the types of antibiotics and biological treatment

systems (Oberoi et al., 2019). Therefore, the urban STPs are considered as one of the main hotspots for the antibiotics released to the environment (Michael et al., 2013; Oberoi et al., 2019). During the biological treatment systems, adsorption and biodegradation are the main pathways for the removal of antibiotics. Tertiary treatment processes can be applied to after the secondary treatment to further reduce organics, turbidity, nitrogen, phosphorus, metals, and pathogens. Most processes involve some type of physiochemical treatment such as coagulation, filtration, activated carbon adsorption of organics, reverse osmosis, and additional disinfection (Brusseau et al., 2019). In the following section, the mechanisms of adsorption and biodegradation, and the effect of some tertiary processes on different classes of antibiotics are discussed.

2.4.1 Antibiotics removal via adsorption

Since antibiotics can be adsorbed to the suspended solid and activated sludge to be remove through sedimentation and disposal of excess sludge, adsorption plays a primary role for the removal of antibiotic in the biological treatment systems (Hörsing et al., 2011). The interation between antibiotics and extracellular polymeric substances (EPS) produced by microbial cells are responsible for the adsorption of antibiotics to the biological sludge, and there are several mechanisms for the interaction, including hydrophobic interaction, cation exchange, cation bridging, surface complexation, hydrogen bond and electrastatic attraction (Oberoi et al., 2019). The affinity of antibiotics sorbed to the biological sludge can be discribed by the solid-liquid partitioning coefficient, K_d (L/kg), which is affected by complex factors, such as the chemical-physical properties of antibiotics and sludge, operational conditions (e.g., biochemical oxygen demand (BOD_5), suspended solid (SS) loading rate, sludge retention time (SRT), hydraulic retention time (HRT), mixed liquor suspended solid (MLSS), pH, and temperature) of the biological treatment systems and so on (Kim et al., 2005). The higher K_d value means the higher sorption of antibiotics to the sludge. Some researchers have determined the K_d values by lab-, pilot- and full-scale studies in different biological treatment systems (Table 2-4), meanwhile the n-octanol/water partitioning coefficient (K_{ow}) was also used to predict the K_d values in some studies (Gerstl, 1990; Huuskonen, 2003; Stevens-Garmon et al., 2011). However, it should be

emphasized that K_{ow} value represents the hydrophobicity of compounds, the prediction based on the K_{ow} value can only be suitable for the non-polar antibiotics, it would not discriminate well between the adsorption properties of polar or charged antibiotics (Michael et al., 2013).

Table 2-4. Summary of the mechanisms and K_d values of antibiotics in previous studies

Antibiotic class	Adsorption mechanisms[1]	Target antibiotics	logK_{ow}[2]	K_d (L/kg)	Sludge systems
β-Lactams	Electrostatic interaction (dominant)	Amoxicillin	0.87	1129 [3]	Activated sludge- hospital sewage
		Ampicillin	1.35	409 [3]	Activated sludge
				44790 [4]	Activated sludge
		Cefalexin	0.65	~0 [4]	Activated sludge
Sulfonamides	Electrostatic interaction (main) Hydrogen bonds Cation exchange Cation bridging Surface complexation	Sulfamethoxazole	0.89	28.6 [5]	Activated sludge (aerobic condition)
				256 [6]	Activated sludge
				11 [7]	Activated sludge (aerobic MBR)
		Sulfadiazine	-0.09	8.3 [4]	Activated sludge
		Sulfamonomethoxine		55.7 [5]	Activated sludge (aerobic condition)
		Sulfadimethoxine	1.63	110.0 [5]	Activated sludge (aerobic condition)
		Sulfapyridine	0.35	295 [6]	Activated sludge
		Sulfamethazine	0.89	100.5 [8]	Activated sludge (sequencing batch reactor treating swine sewage)
Quinolones	Electrostatic interaction Cation exchange Cation bridging	Ciprofloxacin	0.28	4470 [4]	Activated sludge
				1188 [9]	Activated sludge (aerobic condition)
				989 [9]	Activated sludge (nitrifying condition)
				1002 [9]	Activated sludge (anoxic condition)
				19953 [10]	Activated sludge
				20506 [11]	Excess sludge
		Norfloxacin	-1.03	4930 [4]	Activated sludge
				1423 [9]	Activated sludge (aerobic condition)
				1242 [9]	Activated sludge (nitrifying condition)
				1309 [9]	Activated sludge (anoxic condition)
				15849 [10]	Activated sludge
				20302 [11]	Excess sludge
		Ofloxacin	-0.39	984 [9]	Activated sludge (aerobic condition)
				801 [9]	Activated sludge (nitrifying condition)
				888 [9]	Activated sludge (anoxic condition)
				8494 [11]	Excess sludge
		Moxifloxacin	2.9	802 [9]	Activated sludge (aerobic condition)
				578 [9]	Activated sludge (nitrifying condition)
				731 [9]	Activated sludge (anoxic condition)

Quinolones	Electrostatic interaction Cation exchange Cation bridging	Moxifloxacin	2.9	22854 [11]	Excess sludge
		Pipemidic acid	-1.5	1137 [9]	Activated sludge (aerobic condition)
				876 [9]	Activated sludge (nitrifying condition)
				930 [9]	Activated sludge (anoxic condition)
		Piromidic acid	0.42	534 [9]	Activated sludge (aerobic condition)
				414 [9]	Activated sludge (nitrifying condition)
				471 [9]	Activated sludge (anoxic condition)
		Enrofloxacin	0.58	14458 [11]	Excess sludge
		Lomefloxacin	-0.30	10689 [11]	Excess sludge
		Sarafloxacin	0.29	29547 [11]	Excess sludge
Macrolides	Hydrophobic interaction (predominant) Electrostatic interaction (typical pH 6.0-8.0)	Clarithromycin	3.16	262 [6]	Activated sludge
		Azithromycin	3.03	376 [6]	Activated sludge
		Roxithromycin	1.7	51 [7]	MBR aerobic sludge
		Erythromycin	2.6	~0 [4]	Activated sludge
				27.9 [7]	MBR aerobic sludge
Tetracyclines	Electrostatic interaction Cation exchange Cation bridging Surface complexation	Tetracycline	-1.30	22170 [4]	Activated sludge
				8400 [12]	Activated sludge
		Oxytetracycline	-0.90	999 [13]	Activated sludge (aerobic condition)

1. Oberoi et al., 2019; 2. https://www.drugbank.ca/; 3. Kimosop et al., 2016; 4. Li and Zhang, 2010; 5. Yang et al., 2011; 6. Göbel et al., 2005; 7. Polesel et al., 2016; 8. Ben et al., 2014; 9. Dorival-García et al., 2013; 10. Golet et al., 2003; 11. Cao et al., 2019; 12. Kim et al., 2005; 13. Huang et al., 2012

The predominant adorption mechanism for β-lactams is electrosatic interaction. β-Lactam antibiotics were usually detected at a very low concentration in activated sludge (Magee et al., 2018). The most possible reason is that the β-lactam ring is unstable and can be cleaved by β-lactamases, which could be widely produced by bacteria.

Sulfonamides are highly water soluble and difficult to be absorbed to activated sludge, low concentration (<10 μg/kg) of sulfonamides have been detected in activated sludge (Li et al., 2013; Okuda et al., 2009; Peng et al., 2019), which resulting in relatively low removal by absorption in STPs. Since the logK_{OW} values of sulfonamides varies from -0.09 to 1.63 (Table 2-4), they have low potential for hydrophobic partitioning. The electrostatic interactions were doninant for the biosorption of sulfonamides, and they also contain several moieties capble to H-bonding, e.g. $-SO_2^-$ and pyrimidine N as solely H-acceptors, anilinic N and sulfonamidic N as H-acceptors and H-donors. Sulfonamides contain both the amide group ($-NH_3^+$) at pK_{a1} of 1.6-2.6

and the sulfonamide group ($-SO_2NH^-$) at pK_{a2} of 5.0-11.0, which means they can be cationic, neutral and anionic depending on pH, and therefore pH plays an important role on the adsorption of sulfonamides (Oberoi et al., 2019). The surface charge of biological sludge is mainly negative at pH of 3.0-11.0, absorption of sulfonamides shows a decreasing trend with an increasing pH (3.0-11.0) due to electrostatic repulsion. The elimination of sulfapyridine and sulfamethoxazole by absorption were below 6% (Göbel et al., 2007). Adsorption of sulfamethazine in activated sludge decreased with increasing temperature, which revealed that sulfamethazine adsorption was an exothermic process (Ben et al., 2014).

Quinolones have been frequently detected in activated sludge at relatively high concentrations, e.g., norfloxacin (5399 μg/kg dry wt), ofloxacin (2686 μg/kg dry wt), ciprofloxacin (243 μg/kg dry wt), enrofloxacin (10.5 μg/kg dry wt), lomefloxacin (118 μg/kg dry wt) (Li et al., 2013; Okuda et al., 2009). Adsorption has been suggested as the primary pathway for quinolones in biological treatment process, which accounted for 78-84% for ciprofloxacin and norfloxacin (Golet et al., 2003). The logK_{OW} values of quinolones varied from -1.5 to 0.58, except moxifloxacin (logK_{OW} =2.9) (Table 2-4), which means that quinolones are highly hydophilic, while the high adsorption properties could not be simply attributed to hydrophobic interaction. Similar to sulfonamides, quinolones are also amphoteric molecules containing two ionizable functional groups, which could be cationic (pH 4.0-5.0), zwitterionic (pH 7.0-8.0) and anionic (pH>9.0) depending on pH. Therefore, the electrostatic interactions were also doninant for the biosorption of quinolones, and pH plays an important role on the adsorption behaviors (Ferreira et al., 2016; Vasudevan et al., 2009). Zwitterions are the major contributors for the adsoption of quinolones in biological treatment processes, the highest adsorption capacity were usually obtained when the zwittherionic species were prevalent under the pH of 6.0-8.0. In addition, significant correlation of total organic carbon (TOC) and total adsorption concentrations of quinolones was found in Li et al. (2013), implying that the adsorption behaviors of quinolones were significantly depending on the sludge organic contents.

Macrolides were detected in activated sludge at a range from 6.3 μg/kg dry wt (josamycin) to 511.5 μg/kg dry wt (azithromycin) in previous studies, revealed that adsorption plays an important role for macrolides removal in biological treatment

process (Göbel et al., 2005; Hu et al., 2018; Li et al., 2013). Macrolides are sorbed to activated sludge mainly through hydrophobic interactions due to the relatively high logK_{OW} values (1.7-3.16) (Table 2-4). While under the typical pH condition (6.0-8.0), macrolides exist mainly in cationic forms through the protonation of the basic dimethylamino group and the surface of activated sludge is predominantly negatively charged, macrolides could also be sorbed to sludge via electrostatic interactions (Wang et al., 2018). It was found a higher affinity of MBR activated sludge to macrolides than conventional activated sludge, which was attributed to different sludge properties (e.g., EPS properties) (Abegglen et al., 2009). In the moving bed biofilm reactor (MBBR) system, K_d values increased with the increasing biofilm thickness for macrolides (erythromycin, clarithromycin and roxithromycin), possible caused by the higher porosity and accessible surface area in the thickest biofilm (Torresi et al., 2017).

Tetracyclines have been detected in activated sludge in high concentations, for example, 1.667-35.50 mg/kg of oxytetracycline, 0.184-1.908 mg/kg of chlortetracycline, and 0.050-0.466 mg/kg of tetracycline (Ekpeghere et al., 2017; Sun et al., 2016). Despite the low logK_{OW} values of tetracyclines, adsorption is the predominantly pathway for their removal in biological treatment process. Electrostatic interactions and surface complexation have been found to mainly govern the adsorption of tetracyclines in the activated sludge (Hu et al., 2018; Kim et al., 2005). Tetracyclines have complexing properties, which can easily bind to calcium and similar ions, thus forming stable complexes, which can bound to suspended matters or sewage sludge (Drewes et al., 2007). However, some researchers found that tetracyclines may form stong complexes with Ca^{2+} and Mg^{2+}, which might cause the decreased adsorption of tetracyclines to the activated sludge (Figueroa et al., 2005; Li et al., 2010). Both Herry and Freundlich models well fitted the isotherms of tetracyclines, the Herry model constant (K_H) was used as an indicator of the sorption affinity. The specific K_H values of tetracyclines (doxycycline, oxytetracycline and tetracycline) revealed that monovalent cations had the highest sorption affinity, followed by zwitterions, monovalent anions and divalent anions. Under the typical pH conditions in STPs (6.5-7.5), the main contributors to the sorption are zwitterions of tetracycline (96.4-99.1%), oxytetracycline (87.5-98.3%) and doxycycline (97.5-99.6%) (Wang et al., 2018). It has been reported that the adsorption capacity of tetracycline in the sludge reduced by the decreacing SRT

(Kim et al., 2005). K_d of tetracycline decreased with the increasing pH (4.5-8.4), and a gradual decrease trend was found between pH of 6.5 and 8.0 (Li et al., 2010).

2.4.2 Antibiotics removal via biodegradation

As discussed above, adsorption played an important role on the removal of antibiotics. However, adsorption is a phase transfer pathway, which still has potential risk for the antibiotics released into the environment. For some classes of antibiotics, biodegradation is the principal removal pathway. Biodegradation is a process that can breakdown the complex compounds through biotransformation to metabolic intermediates or through complete mineralization to CO_2 and H_2O (Alvarino et al., 2016). Different intermediates could be formed by either breakdown of the parent antibiotics or without breakdown of parent compounds, such as hydroxylation, acetylation of the amino group, demethylation and so on (Larcher et al., 2011; Nguyen et al., 2018). Microorganisms in the activated sludge could degrade antibiotics through using them as carbon and energy source or via cometabolism, which would involve the related functional enzymes and genes produced by functional microorganisms (Wright et al., 2005; Jia et al., 2018; Ma et al., 2020). Furthermore, there are also some operational factors that could influence the biodegradation of antibiotics, e.g., BOD_5, SS, HRT, SRT, MLSS, pH, temperature, and food-microorganism ratio (F/M ratio).

β-Lactams are relatively easily eliminated in biological treatment mainly due to their chemically unstable β-lactam ring, which is susceptible to environmental conditions (e.g., pH, temperature, light and so on). β-Lactam antibiotics were mainly transformed via the hydrolysis of β-lactam ring by β-lactamases even under ambient pH and temperature conditions, and the transformation products could be the final products or be further degraded (Mayers, 2009). It was reported that piperacillin could be transformed to hydrolyzed piperacillin as main product and bis-hydrolyzed piperacillin as minor product of abiotic transformation products, which is a dead-end transformation product (Längin et al., 2009). While for amoxicillin, amoxicillin diketopiperacine-2',5' and amoxilloic acid diastereomers were main transformation products which were frequently detected in the STPs effluent, these transformation products were

demonstrated to be further mineralized during biological treatment (Längin et al., 2009; Lamm et al., 2009; Hirte et al., 2016; Pérez-Parada et al., 2011).

Sulfonamides are difficult to be absorbed to the activated sludge as discussed above, however, biodegradation of sulfonamides have been detected in both pure and mixed cultures under different conditions (aerobic, anoxic and anaerobic) (García-Galán et al., 2011; Jia et al., 2017; Larcher and Yargeau, 2011; Mao et al., 2018; Majewsky et al., 2015; Nödler et al., 2012). The removal efficiency of sulfonamides in the secondary treatment varied from <0 to 100% in the previous studies (Yang et al., 2005; Yu et al., 2009). The transformation products of sulfonamides can be classified into (1) products that undergo transformation (such as hydroxylation, acetylation, nitation and so on) where the parent is not decomposed, and (2) breakdown products with or without transformation resulting from the parent compound cleavage (Majewsky et al., 2015). Sulfamethoxazole is the most frequently detected sulfonamides in the aquatic environment, it has been reported that *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Rhodococcus equi*, *Rhodococcus erythropolis*, *Rhodococcus rhodocrous*, and *Rhodococcus zopfii* exist in STP activated sludge have the ability to degrade sulfamethoxazole, however, the biodegradation ability of pure culture decreased when they were mixed with other microorganisms (Larcher and Yargeau, 2011). Under aerobic condition, 3-amino-5-methyl-isoxazole was the main transformation products of sulfamethoxazole (Mao et al., 2018); while under denitrifying conditions, 4-nitro-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (*4-nitro-sulfamethoxazole*) and N-(5-methylisoxazol-3-yl)-benzenesulfonamide (*desamino-sulfamethoxazole*) were produced as the intermediates (Nödler et al., 2012). It indicates that there might be different transformation pathways of sulfonamides under different conditions. It is worth noting that N_4-acetylsulfamethoxazole as a main metabolite of sulfamethoxazole could be excreted from human bodies, accounting for more than 50% of the dose, it could be degraded during the secondary treatment and could also de-conjugate into sulfamethoxazole, resulting in the underestimation of removal efficiency on sulfamethoxazole (Göbel et al., 2007). In addition, it has been reported that a cluster of genes encoding two monooxygenases (SadA and SadB) and one FMN reductase (SadC) which could inactivate sulfonamides (Ricken et al., 2017). SadA and SadC are

responsible for the initial attack of sulfonamide molecules resulting in the release of 4-aminophenol, which could be further transformed into 1,2,4-trihydroxybenzene by SadB and SanC prior to mineralization (Richen et al., 2017). Higher temperature can promote the endothermic hydrolysis reaction and improve biodegradation of sulfonamides, thus the removal efficiencies of sulfonamides in summer were higher than that in winter (Dan et al., 2013). However, higher SRT was not found to increase the removal efficiency of sulfamethoxazole and sulfapyridine (Göbel et al., 2007).

Quinolones were mainly removed through adsorption during the secondary treatment as discussed above. It has been reported that quinolones are resistant to hydrolysis and highly recalcitrant to biodegradation in previous study (Babić et al., 2013; Janecko et al., 2016). However, there are still some studies reported on the biodegradation of quinolone antibiotics under aerobic, anoxic and anaerobic conditions. A combination of three bacterial strains (*Labrys portucalensis* F11, *Rhodococcus* sp. FP1 and *Rhodococcus* sp. S2), are capable to degrade a serial fluoroaromatic compounds, were used to test the biodegradation of four quinolones (ofloxacin, norfloxacin, ciprofloxacin and moxifloxacin) under aerobic condition, comparing with the biodegradation efficiency in STPs, the bacterial consortium was able to degrade these quinolones in aerobic condition into a higher extent in an individual assay (Maia et al., 2014). Terzic et al. (2011) investigated the biodegradation of ciprofloxacin and norfloxacin in a membrane bioreactor and identified two types of transformation by-products via N-succinylation (*N*-succinyl-ciprofloxacin) and decomposition of piperazine ring form derivatives where piperazine moiety was replaced by a 7-[(2-carboxymethyl) amino] group (7-[(carboxymethyl)amino]-1cyclopropyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid). In the anaerobic fixed bed biofilm reactors, the biodegradation of ciprofloxacin was derived by hydrogenotrophic methanogens (mainly *Methanobacterium* genus) (Carneiro et al., 2020). Jia et al. (2018) examined the biodegradation of ciprofloxacin in an anaerobic sulfidogenic condition, and the results suggested that ciprofloxacin was biodegraded via hydroxylation reaction catalyzed by cytochrome P450 enzyme and demethylation reaction in piperazinyl ring, which resulting in the production of six predominant intermediates. In the denitrifying condition, C_2H_2 having a piperazinyl substituent of ciprofloxacin is removed by

demethylations to generate CIP-BBP1 and transferred to CIP-BBP4 by loss of the C_2H_5N fragment of CIP-BBP1 (Hassan et al., 2020). The enzymes involved in the biodegradation of quinolones are largely unknow, however, some previous studies identified that both cytochrome P450 enzyme and ligninolytic enzymes Lac play a role in the biotransformation of quinolones (Jia et al., 2018; Gao et al., 2018; Prieto et al., 2011). Additionally, it has been reported that the organic loading rate in the influent negatively influence the biodegradation of ciprofloxacin (Carneiro et al., 2020).

Erythromycin, clarithromycin, azithromycin and roxithromycin are highly used macrolides worldwide, and they could be degraded in the secondary treatment by a variable rate (10-80%) (Göbel et al., 2007). It should be notice that the removal of erythromycin was faster than other macrolides, and some studies also indicated that erythromycin could be efficiently biotransformed both under aerobic and anaerobic conditions (Kwon, 2016; Terzic et al., 2018). Phosphorylation is a well-known pathway for the biotransformation of macrolides in the activated sludge system, and the phosphorylated transformation products of clarithromycin, azithromycin, roxithromycin and erythromycin have been identified in the STPs effluent (Senta et al., 2017; Terzic et al., 2011). Some other biotransformation products of clarithromycin and azithromycin, such as 14-hydroxy clarithromycin, *N*-dimethyl clarithromycin and descladinosyl azithromycin, were also observed in both municipal and industrial wastewater systems (Ibáñez et al., 2017; Senta et al., 2017). Senta et al. (2017) reported three proposed biotransformation pathways of azithromycin in an enriched culture system, which involved enzymatic hydrolytic opening of the macrolactone ring, cleavage of the desosamine and cladinose moiety, and oxidation of the hydroxy group and so on. For the biotransformation of erythromycin, most of the products were formed via consecutive enzymatic cleavage of the cladinose and desosamine units, and these products are more stable than the parent compounds; while most of the biotransformation products of clarithromycin were formed by the phosphorylation of desosamine and enzymatic hydrolysis of the macrolactone ring (Senta et al., 2017). It was observed that higher SRTs could enhance the eliminant of clarithromycin and erythromycin-H_2O (Göbel et al., 2007).

Tetracycline antibiotics have complexing properties and are poorly biodegradable,

sorption was found to be the principal removal mechanism in activated sludge (Kim et al., 2005). Some researchers studied the degradation of tetracyclines by chemical processes (Jiao et al., 2008; Chen et al., 2017), however, little is known about their biodegradation. Some pure cultures of bacterial strains have been reported to be capable of degrading tetracycline (Ghosh et al., 2009; Volkers et al., 2010; Leng et al., 2016). A tetracycline resistant, aerobic *Sphingobacterium* sp. strain PM2-P1-29 was characterized to harbor a *tet*(X) gene, which encodes for a NADP-dependentmonooxygenase that requires oxygen to degrade tetracycline (Ghosh et al., 2009). The flavin-dependent monooxygenase TetX2 from *Bacteroides thetaiotaomicron* could degrade tetracycline to 11a-hydroxy-tetracyclines via hydroxylation (Volkers et al., 2010). Leng et al. (2016) isolated *Stenotrophomonas maltophilia* strain DT1 which is capable of degrading tetracycline, and six possible biotransformation products were identified, *N*-methyl, carbonyl, and amine groups were removed during biotransformation. Furthermore, Shi et al. (2011) obtained that the nitrifying granular sludge system showed outstanding biodegradation ability of tetracyclines compared to conventional activated sludge system, and the removal of tetracycline could be enhanced in the presence of easily biodegradable substrates. Taşkan et al. (2016) firstly reported that *Betaproteobacteria* is active in a H_2-based membrane biofilm reactor (MBfR) in degrading and mineralizing tetracycline, and H_2-MBfR could be one of the alternative methods against chemical methods. Moreover, there are three transformation products: 4-epitetracycline, anhydrotetracycline, and epianhydrotetracycline, and 4-epitetracycline was found to be dominant in the H_2-MBfR effluent (Taşkan et al., 2016). In addition, some enzymes produced by fungi, such as laccase and lignin peroxidase, have been identified to degrade tetracyclines (Llorca et al., 2015; Wen et al., 2009).

2.5 Model development

The release of untreated antibiotics from STPs is the main route for human-used antibiotics entering into the aquatic environment. The discharge of antibiotics from STPs varied by many factors, e.g., types of antibiotics, location, season and so on. However, monitoring of antibiotics is restricted by the available analytical methods in laboratories, cost and time consumption. Some researchers developed several models to

predict the volumes of organic micropollutants entering into STPs (Carballa et al., 2008, Ort et al., 2009; Verlicchi et al., 2014), the removal of organic micropollutants in each individual unit of STPs (Polesel et al, 2016; Pomies et al., 2013; Taboada-Santos et al., 2020), which also involved the estimation of some important parameters of organic micropollutants in STPs, e.g., sorption distribution coefficients (Stevens-Garmon et al., 2011; Hyland et al., 2012; Lakshminarasimman et al., 2018), biodegradation rate constant (Joss et al., 2006; Park et al., 2016; Suarez et al., 2010) and so on. However, since the concepts varied among the predictive models in each part, there is presently no integrate of these models to predict the release of antibiotics from consumption data.

2.5.1 Predicted environmental concentration of the STP influent

In predicting the concentrations of human-used antibiotics in sewage influent, three relevant factors should be considered (Carballa et al., 2008, Ort et al., 2009; Verlicchi et al., 2014): 1) human-used antibiotics consumption; 2) excretion rate of each antibiotic from human bodies; 3) wastewater produced volume per inhabitant in studied aera. The antibiotics consumption is an essential factor influencing the accuracy of predictive models, and it could be calculated from several data sources, e.g., prescription volumes from hospitals and clinics, shipping volumes of antibiotics in the market, or sales volumes from pharmaceutical companies. After metabolism of antibiotics in human bodies, part of the parent compounds and metabolites are excreted to the sewer system. Hence, the excretion rate of each antibiotic, defined as the percentage of individual antibiotic excreted as the unchanged compound (parent compound), is also an important factor affecting the concentration of antibiotics entering into the STPs. Because the antibiotics and their metabolites would be excreted to the sewer system and diluted by the wastewater, the wastewater produced volume per inhabitant would also affect the concentration of antibiotics in the sewage influent. Among the three relevant factors, antibiotics consumption and excretion rate of each antibiotic are two uncertain factors affecting the predictive accuracy seriously. For the consumption of antibiotics, no matter which data source was chosen to calculate, it could not accurately represent the actual amounts of antibiotics consumption due to the limitation of data statistic and the gap between the prescription volumes and actual usage volumes (Azuma et al., 2016;

Verlicchi et al., 2013). Therefore, how to choose the data source become an important point during the prediction. Moreover, the excretion rates of antibiotics exhibit a range of variations by human age, gender and so on, which could affect the metabolism of antibiotics in human bodies. It is impossible to choose an amount representing the accurate average excretion rate of actual amount in studied area, which would reduce the accuracy of predictive models.

2.5.2 Predicting the fate of antibiotics in STPs

Plant-wide simulation has been successfully applied in the energy consumption and economic aspects of STPs, which inspired some researchers to apply it to assess the fate of organic micropollutants in STPs (Polesel et al, 2016; Pomies et al., 2013; Taboada-Santos et al., 2020). There are several models developed for individual units separately, e.g., primary sedimentation unit (Takács et al., 1991; Carballa et al., 2008), activated sludge unit (Alvarino et al., 2014; Guo and Vanrolleghem, 2014), sludge thickening and dewatering unit (Gernaey et al., 2014), and anaerobic sludge digester unit (Batstone et al., 2002, Taboada-Santos et al., 2019). All of the models involved in each unit based on the concept of mass balance.

In the physic-chemical separation units, like primary sedimentation tank, chemically enhanced primary treatment, and rotating belt filters, the models assumed that there is no biodegradation occurred and the removal of organic micropollutants is attributed to the solid separation, which could be modelled on the basis of the gravity settling principle (Takács et al., 1991). During these units, the sorption distribution coefficient of organic micropollutants was an important factor, which were measured and estimated by many previous studies (Carballa et al., 2008; Stevens-Garmon et al., 2011; Hyland et al., 2012).

During the biological treatment units, such as conventional activated sludge reactor, membrane bioreactor and so on, both biodegradation and sorption were considered as main removal pathways. The biodegradation of organic micropollutants were modelled by the biodegradation rate constants, which could be estimated by the batch experiments, and the first-order kinetics was the most popular model applying to estimate the rate constants (Joss et al., 2006; Park et al., 2016; Suarez et al., 2010).

In the sludge thickening and dewatering units, the models assumed that there is no variation in neither soluble nor sorbed concentration and just used a constant thickening or dewatering factor (Gernaey et al., 2014).

For the anaerobic sludge digester unit, the fate of organic micropollutants was modelled by a fixed biodegradability instead of the first-order kinetics in the mainstream biological units (Taboada-Santos et al., 2019). Because it has been found that the biodegradation of organic micropollutants in anaerobic digester unit is limited by thermodynamic rather than kinetic constraints, and it would result in the overestimation of the biodegradation if the pseudo-first order kinetics were applied (Gonzalez-Gil et al., 2018).

2.6 Summary

In this Chapter, the review of the analytical methods of antibiotics, consumption, occurrence and potential risk in Japan, the removal pathway of antibiotics in STPs, and also related models were summarized. The findings are as follows:

- ✓ The analytical methods of antibiotics have been developed in different environmental matrices; however, it is difficult to establish a multi-target method to detect all antibiotics simultaneously due to the variations of chemical and physical properties of antibiotics from different classes.
- ✓ Because of the ecotoxicity and antibiotic resistance resulted from the consumption and release of antibiotics, and STPs were as hotpots of the release of human-used antibiotics, it is essential to study the occurrence of antibiotics in the environment and removal mechanisms of antibiotics in STPs for further risk assessment.
- ✓ Although many predictive models developed by previous researchers for the release of human-used antibiotics, there is presently no integrate of these models to predict the release of antibiotics from consumption data due to the various concepts. Therefore, it is essential to propose the integrate models suitable for different processes.
- ✓ Previous risk assessment work of antibiotic residues only considered the ecotoxicity, it is urgent to involve the potential risk on the selection and development of antibiotic resistance for antibiotic residues, based on which to propose the discharge

limits of antibiotics.

2.7 Reference

Abegglen, C., Joss, A., McArdell, C. S., Fink, G., Schlüsener, M. P., Ternes, T. A., Siegrist, H., 2009. The fate of selected micropollutants in a single-house MBR. *Water Research*, *43*(7), pp.2036-2046.

Abuin S, Codony R, Compano R, Granados M, Prat MD., 2006. Analysis of macrolide antibiotics in river water by solid-phase extraction and liquid chromatography-mass spectrometry. *J Chromatogr A 1114*, pp.73-81.

Adachi, F., Yamamoto, A., Takakura, K.I., Kawahara, R., 2013. Occurrence of fluoroquinolones and fluoroquinolone-resistance genes in the aquatic environment. *Science of the Total Environment*, *444*, pp.508-514.

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology*, *8*(4), pp.251-259.

Alvarino, T., Nastold, P., Suarez, S., Omil, F., Corvini, P.F.X., Bouju, H., 2016. Role of biotransformation, sorption and mineralization of 14C-labelled sulfamethoxazole under different redox conditions. *Science of The Total Environment*, *542*, pp.706-715.

Alvarino, T., Suarez, S., Lema, J. M., Omil, F., 2014. Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. *Journal of Hazardous Materials*, *278*, pp.506-513.

Alygizakis, N.A., Gago-Ferrero, P., Borova, V.L., Pavlidou, A., Hatzianestis, I., Thomaidis, N.S., 2016. Occurrence and spatial distribution of 158 pharmaceuticals, drugs of abuse and related metabolites in offshore seawater. *Science of the Total Environment*, *541*, pp.1097-1105.

Anderson, C.R., Rupp, H.S., Wu, W.H., 2005. Complexities in tetracycline analysis—chemistry, matrix extraction, cleanup, and liquid chromatography. *Journal of Chromatography A*, *1075*(1-2), pp.23-32.

Arpin-Pont, L., Bueno, M. J. M., Gomez, E., Fenet, H., 2016. Occurrence of PPCPs in the marine environment: a review. *Environmental Science and Pollution Research*, *23*(6), pp.4978-4991.

Asai, T., Kojima, A., Harada, K., Ishihara, K., Takahashi, T. and Tamura, Y., 2005. Correlation between the usage volume of veterinary therapeutic antimicrobials and resistance in Escherichia coli isolated from the feces of food-producing animals in Japan. *Japanese journal of infectious diseases, 58*(6), pp.369.

Azuma, T., Nakada, N., Yamashita, N., Tanaka, H., 2015. Evaluation of concentrations of pharmaceuticals detected in sewage influents in Japan by using annual shipping and sales data. *Chemosphere*, *138*, pp.770-776.
Azuma, T., Nakada, N., Yamashita, N., Tanaka, H., 2015. Evaluation of concentrations of pharmaceuticals detected in sewage influents in Japan by using annual shipping and sales data. *Chemosphere*, *138*, pp.770-776.
Baalbaki, Z., Torfs, E., Yargeau, V., Vanrolleghem, P.A., 2017. Predicting the fate of micropollutants during wastewater treatment: Calibration and sensitivity analysis. *Science of the Total Environment*, *601*, pp.874-885.

Babić, S., Periša, M., Škorić, I., 2013. Photolytic degradation of norfloxacin, enrofloxacin and ciprofloxacin in various aqueous media. *Chemosphere*, *91*(11), pp.1635-1642.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W.T.M., Siegrist, H., Vavilin, V. A., 2002. The IWA anaerobic digestion model no 1 (ADM1). *Water Science and Technology*, *45*(10), pp.65-73.

Ben, W., Qiang, Z., Yin, X., Qu, J., Pan, X., 2014. Adsorption behavior of sulfamethazine in an activated sludge process treating swine wastewater. *Journal of Environmental Sciences*, *26*(8), pp.1623-1629.
Ben, W., Qiang, Z., Yin, X., Qu, J., Pan, X., 2014. Adsorption behavior of sulfamethazine in an activated sludge process treating swine wastewater. *Journal of Environmental Sciences*, *26*(8), pp.1623-1629.
Bronzwaer, S.L., Cars, O., Buchholz, U., Mölstad, S., Goettsch, W., Veldhuijzen, I.K., Kool, J.L., Sprenger, M.J. and Degener, J.E., 2002. The relationship between antimicrobial use and antimicrobial resistance in Europe. *Emerging infectious diseases, 8*(3), pp.278.

Brusseau, M. L., Pepper, I. L., Gerba, C., 2019. *Environmental and pollution science*. Academic press.

Buchberger, W.W., 2007. Novel analytical procedures for screening of drug residues in water, waste water, sediment and sludge. *Analytica Chimica Acta*, *593*(2), pp.129-139.

Caldas, S.S., Goncalves, F.F., Primel, E.G., Prestes, O.D., Martins, M.L., Zanella, R., 2011. Modern techniques of sample preparation for pesticide residues determination in water by liquid chromatography with detection by diode array and mass spectrometry. *Quimica Nova*, *34*(9), pp.1604-1617.

Cao, D. Q., Yang, W. Y., Wang, Z., Hao, X. D., 2019. Role of extracellular polymeric substance in adsorption of quinolone antibiotics by microbial cells in excess sludge. *Chemical Engineering Journal*, *370*, pp.684-694.

Carballa, M., Fink, G., Omil, F., Lema, J. M., Ternes, T., 2008. Determination of the solid-water distribution coefficient (K_d) for pharmaceuticals, estrogens and musk fragrances in digested sludge. *Water Research*, *42*(1-2), pp.287-295.

Carballa, M., Omil, F., Lema, J. M., 2008. Comparison of predicted and measured concentrations of selected pharmaceuticals, fragrances and hormones in Spanish sewage. *Chemosphere*, *72*(8), pp.1118-1123.

Carneiro, R. B., Mukaeda, C. M., Sabatini, C. A., Santos-Neto, Á. J., Zaiat, M., 2020. Influence of organic loading rate on ciprofloxacin and sulfamethoxazole biodegradation in anaerobic fixed bed biofilm reactors. *Journal of Environmental Management*, *273*, pp.111170.

Carvalho, I.T. and Santos, L., 2016. Antibiotics in the aquatic environments: a review of the European scenario. *Environment International, 94*, pp.736-75.

Chang, H., Hu, J., Asami, M., Kunikane, S., 2008. Simultaneous analysis of 16 sulfonamide and trimethoprim antibiotics in environmental waters by liquid chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography A*, *1190*(1-2), pp.390-393.

Chen, Y. Y., Ma, Y. L., Yang, J., Wang, L. Q., Lv, J. M., Ren, C. J., 2017. Aqueous tetracycline degradation by H2O2 alone: removal and transformation pathway. *Chemical Engineering Journal*, *307*, pp.15-23.

Chitescu, C.L., Kaklamanos, G., Nicolau, A.I., Stolker, A.A.M.L., 2015. High sensitive multiresidue analysis of pharmaceuticals and antifungals in surface water using U-HPLC-Q-Exactive Orbitrap HRMS. Application to the Danube river basin on the Romanian territory. *Science of the total environment*, *532*, pp.501-511.

Christian, T., Schneider, R.J., Färber, H.A., Skutlarek, D., Meyer, M.T., Goldbach, H.E., 2003. Determination of antibiotic residues in manure, soil, and surface waters. *Acta hydrochimica et hydrobiologica*, *31*(1), pp.36-44.

Dan, A., Yang, Y., Dai, Y. N., Chen, C. X., Wang, S. Y., Tao, R., 2013. Removal and factors influencing removal of sulfonamides and trimethoprim from domestic sewage in constructed wetlands. *Bioresource technology*, *146*, pp.363-370.

De García, S. A. O., Pinto, G. P., García-Encina, P. A., Irusta-Mata, R., 2014. Ecotoxicity and environmental risk assessment of pharmaceuticals and personal care products in aquatic environments and wastewater treatment plants. *Ecotoxicology*, *23*(8), pp.1517-1533.

De García, S.A.O., Pinto, G.P., García-Encina, P.A., Irusta-Mata, R., 2014. Ecotoxicity and environmental risk assessment of pharmaceuticals and personal care products in aquatic environments and wastewater treatment plants. *Ecotoxicology*, *23*(8), pp.1517-1533.

Deshpande, A.D., Baheti, K.G., Chatterjee, N.R., 2004. Degradation of β-lactam antibiotics. *Current science*, pp.1684-1695.

Deshpande, A.D., Baheti, K.G., Chatterjee, N.R., 2004. Degradation of β-lactam antibiotics. *Current science*, pp.1684-1695.

Dinh, Q.T., Alliot, F., Moreau-Guigon, E., Eurin, J., Chevreuil, M., Labadie, P., 2011. Measurement of trace levels of antibiotics in river water using on-line enrichment and triple-quadrupole LC–MS/MS. *Talanta*, *85*(3), pp.1238-1245.

Dorival-García, N., Zafra-Gómez, A., Navalón, A., González-López, J., Hontoria, E., Vílchez, J. L., 2013. Removal and degradation characteristics of quinolone antibiotics in laboratory-scale activated sludge reactors under aerobic, nitrifying and anoxic conditions. *Journal of environmental management*, *120*, pp.75-83.

Drewes, J. E., 2007. Removal of pharmaceutical residues during wastewater treatment. *Comprehensive Analytical Chemistry*, *50*, pp.427-449.

ECDC, 2019. Antimicrobial consumption interactive database (ESAC-Net). https://www.ecdc.europa.eu/en/antimicrobial-consumption/surveillance-and-disease-data/database

Eguchi, K., Nagase, H., Ozawa, M., Endoh, Y.S., Goto, K., Hirata, K., Miyamoto, K., Yoshimura, H., 2004. Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. *Chemosphere, 57*(11), pp.1733-1738.

EMEA, 2018. Guideline on the environmental risk assessment of medicinal products for human use (draft). Available from: https://www.ema.europa.eu/documents/scientific-guideline/draft-guideline-environmental-risk-assessment-medicinal-products-human-use-revision-1_en.pdf (accessed 25 June 2019).

Farouk, F., Azzazy, H.M., Niessen, W.M., 2015. Challenges in the determination of aminoglycoside antibiotics, a review. *Analytica chimica acta*, *890*, pp.21-43.

Feitosa-Felizzola, J., Chiron, S., 2009. Occurrence and distribution of selected antibiotics in a small Mediterranean stream (Arc River, Southern France). *Journal of Hydrology*, *364*(1-2), pp.50-57.

Feitosa-Felizzola, J., Chiron, S., 2009. Occurrence and distribution of selected antibiotics in a small Mediterranean stream (Arc River, Southern France). *Journal of Hydrology*, *364*(1-2), pp.50-57.

Ferreira, V.R., Amorim, C.L., Cravo, S.M., Tiritan, M.E., Castro, P.M., Afonso, C.M., 2016. Fluoroquinolones biosorption onto microbial biomass: activated sludge and aerobic granular sludge. *International Biodeterioration & Biodegradation*, *110*, pp.53-60.

Figueroa, R. A., MacKay, A. A., 2005. Sorption of oxytetracycline to iron oxides and iron oxide-rich soils. *Environmental Science & Technology*, *39*(17), pp.6664-6671.

Gao, N., Liu, C. X., Xu, Q. M., Cheng, J. S., Yuan, Y. J., 2018. Simultaneous removal of ciprofloxacin, norfloxacin, sulfamethoxazole by co-producing oxidative enzymes system of Phanerochaete chrysosporium and Pycnoporus sanguineus. *Chemosphere*, *195*, pp.146-155.

Gao, S., Zhang, Z.P., Karnes, H.T., 2005. Sensitivity enhancement in liquid chromatography/atmospheric pressure ionization mass spectrometry using derivatization and mobile phase additives. *Journal of Chromatography B*, *825*(2), pp.98-110.

García-Galán, M. J., Rodríguez-Rodríguez, C. E., Vicent, T., Caminal, G., Díaz-Cruz, M. S., Barceló, D., 2011. Biodegradation of sulfamethazine by Trametes versicolor: Removal from sewage sludge and identification of intermediate products by UPLC–QqTOF-MS. *Science of the Total Environment*, *409*(24), pp.5505-5512.

García-Galán, M.J., Díaz-Cruz, M.S., Barceló, D., 2011. Occurrence of sulfonamide residues along the Ebro river basin: removal in wastewater treatment plants and environmental impact assessment. *Environment international*, *37*(2), pp.462-473.

Gernaey, K. V., Jeppsson, U., 2014. *Benchmarking of control strategies for wastewater treatment plants*. IWA publishing.

Gerstl, Z., 1990. Estimation of organic chemical sorption by soils. *Journal of contaminant hydrology*, *6*(4), pp.357-375.

Ghosh, S., Sadowsky, M. J., Roberts, M. C., Gralnick, J. A., LaPara, T. M., 2009. Sphingobacterium sp. strain PM2-P1-29 harbours a functional tet (X) gene encoding for the degradation of tetracycline. *Journal of Applied Microbiology*, *106*(4), pp.1336-1342.

Gobel A, Thomsen A, McArdell CS, Alder AC, Giger W, Theiss N, Loffler D, Ternes TA., 2005. Extraction and determination of sulfonamides, macrolides, and trimethoprim in sewage sludge. *J Chromatogr A 1085,* pp.179-189.

Golet, E. M., Xifra, I., Siegrist, H., Alder, A. C., Giger, W., 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environmental science & technology*, *37*(15), pp.3243-3249.

Golet, E. M., Xifra, I., Siegrist, H., Alder, A. C., Giger, W., 2003. Environmental exposure assessment of fluoroquinolone antibacterial agents from sewage to soil. *Environmental Science & Technology*, *37*(15), pp.3243-3249.

Gonzalez-Gil, L., Mauricio-Iglesias, M., Carballa, M., Lema, J. M., 2018. Why are organic micropollutants not fully biotransformed? A mechanistic modelling approach to anaerobic systems. *Water Research*, *142*, pp.115-128.

Grenni, P., Ancona, V., Caracciolo, A.B., 2018. Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal*, *136*, pp.25-39.

Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. *Journal of Chromatography A*, *1248*, pp.104-121.

Guo, L., Vanrolleghem, P. A., 2014. Calibration and validation of an activated sludge model for greenhouse gases no. 1 (ASMG1): prediction of temperature-dependent N 2 O emission dynamics. *Bioprocess and Biosystems Engineering*, *37*(2), pp.151-163.

Göbel, A., McArdell, C. S., Joss, A., Siegrist, H., Giger, W., 2007. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of the Total Environment*, *372*(2-3), pp.361-371.

Göbel, A., Thomsen, A., McArdell, C. S., Joss, A., Giger, W., 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. *Environmental science & technology*, *39*(11), pp.3981-3989.

Halling-Sørensen, B., 2000. Algal toxicity of antibacterial agents used in intensive farming. *Chemosphere, 40*(7), pp.731-739.

Halling-Sørensen, B., Jensen, J., Tjørnelund, J., Montforts, M. H. M. M., 2001. Worst-case estimations of predicted environmental soil concentrations (PEC) of selected veterinary antibiotics and residues used in Danish agriculture. In *Pharmaceuticals in the Environment* (pp. 143-157). Springer, Berlin, Heidelberg.

Hassan, M., Zhu, G., Yang, Z., Lu, Y., Lang, Y., Gong, L., Shan, H., 2020. Effect of the C/N Ratio on Biodegradation of Ciprofloxacin and Denitrification from Low C/N Wastewater as Assessed by a Novel 3D-BER System. *Sustainability*, *12*(18), pp.7611.

Hemström, P., Irgum, K., 2006. Hydrophilic interaction chromatography. *Journal of separation science*, *29*(12), pp.1784-1821.

Hirte, K., Seiwert, B., Schüürmann, G., Reemtsma, T., 2016. New hydrolysis products of the β-lactam antibiotic amoxicillin, their pH-dependent formation and search in municipal wastewater. *Water Research*, *88*, pp.880-888.

Horie, M., Saito, K., Ishii, R., Yoshida, T., Haramaki, Y., Nakazawa, H., 1998. Simultaneous determination of five macrolide antibiotics in meat by high-performance liquid chromatography. *Journal of Chromatography A*, *812*(1-2), pp.295-302.

Hu, J., Zhou, J., Zhou, S., Wu, P., Tsang, Y. F., 2018. Occurrence and fate of antibiotics in a wastewater treatment plant and their biological effects on receiving waters in Guizhou. *Process Safety and Environmental Protection*, *113*, pp. 483-490.

Huang, M. H., Yang, Y. D., Chen, D. H., Chen, L., Guo, H. D., 2012. Removal mechanism of trace oxytetracycline by aerobic sludge. *Process Safety and Environmental Protection*, *90*(2), pp.141-146.

Huuskonen, J., 2003. Prediction of soil sorption coefficient of a diverse set of organic chemicals from molecular structure. *Journal of chemical information and computer sciences*, *43*(5), pp.1457-1462.

Hyland, K. C., Dickenson, E. R., Drewes, J. E., Higgins, C. P., 2012. Sorption of ionized and neutral emerging trace organic compounds onto activated sludge from different wastewater treatment configurations. *Water Research*, *46*(6), pp.1958-1968.

Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., la Cour Jansen, J., Andersen, H. R., 2011. Determination of sorption of seventy-five pharmaceuticals in sewage sludge. *Water research*, *45*(15), pp.4470-4482.

Ibáñez, M., Borova, V., Boix, C., Aalizadeh, R., Bade, R., Thomaidis, N. S., Hernández, F., 2017. UHPLC-QTOF MS screening of pharmaceuticals and their metabolites in treated wastewater samples from Athens. *Journal of Hazardous Materials*, *323*, pp.26-35.

Jacobsen, A. M., Halling-Sørensen, B., 2006. Multi-component analysis of tetracyclines, sulfonamides and tylosin in swine manure by liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *384*(5), pp.1164-1174.

Jacobsen, A. M., Halling-Sørensen, B., Ingerslev, F., Hansen, S. H., 2004. Simultaneous extraction of tetracycline, macrolide and sulfonamide antibiotics from agricultural soils using pressurised liquid extraction, followed by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *1038*(1-2), pp.157-170.

Jacobsen, A.M., Halling-Sørensen, B., Ingerslev, F., Hansen, S.H., 2004. Simultaneous extraction of tetracycline, macrolide and sulfonamide antibiotics from agricultural soils using pressurised liquid extraction, followed by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *1038*(1-2), pp.157-170.

Janecko, N., Pokludova, L., Blahova, J., Svobodova, Z., Literak, I., 2016. Implications of fluoroquinolone contamination for the aquatic environment—A review. *Environmental Toxicology and Chemistry*, *35*(11), pp.2647-2656.

Jia, Y., Khanal, S. K., Shu, H., Zhang, H., Chen, G. H., Lu, H., 2018. Ciprofloxacin degradation in anaerobic sulfate-reducing bacteria (SRB) sludge system: mechanism and pathways. *Water Research*, *136*, pp.64-74.

Jia, Y., Khanal, S. K., Shu, H., Zhang, H., Chen, G. H., Lu, H., 2018. Ciprofloxacin degradation in anaerobic sulfate-reducing bacteria (SRB) sludge system: mechanism and pathways. *WaterR*, *136*, pp.64-74.

Jia, Y., Khanal, S.K., Zhang, H., Chen, G.H., Lu, H., 2017. Sulfamethoxazole degradation in anaerobic sulfate-reducing bacteria sludge system. *Water research*, *119*, pp.12-20.

Jiao, S., Zheng, S., Yin, D., Wang, L., Chen, L., 2008. Aqueous photolysis of tetracycline and toxicity of photolytic products to luminescent bacteria. *Chemosphere*, *73*(3), pp.377-382.

Jiménez-Lozano, E., Roy, D., Barrón, D., Barbosa, J., 2004. Effective sorbents for solid-phase extraction in the analysis of quinolones in animal tissues by capillary electrophoresis. *Electrophoresis*, *25*(1), pp.65-73.

Jones, O.A.H., Voulvoulis, N., Lester, J.N., 2002. Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. *Water research*, *36*(20), pp.5013-5022.

Jones, O.A.H., Voulvoulis, N., Lester, J.N., 2005. Human pharmaceuticals in wastewater treatment processes. *Critical reviews in environmental science and technology*, *35*(4), pp.401-427.

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. *Water Research*, *40*(8), pp.1686-1696.

Kachhawaha, A. S., Nagarnaik, P. M., Labhasetwar, P., Banerjee, K., 2020. A review of recently developed LC-MS/MS methods for the analysis of pharmaceuticals and personal care products in water. *Journal of AOAC International*, *103*(1), pp.9-22.

Kahsay, G., Song, H., Van Schepdael, A., Cabooter, D., Adams, E., 2014. Hydrophilic interaction chromatography (HILIC) in the analysis of antibiotics. *Journal of pharmaceutical and biomedical analysis*, *87*, pp.142-154.

Kanfer, I., Skinner, M.F., Walker, R.B., 1998. Analysis of macrolide antibiotics. *Journal of Chromatography A*, *812*(1-2), pp.255-286.

Kaufmann, A., Maden, K., 2005. Determination of 11 aminoglycosides in meat and liver by liquid chromatography with tandem mass spectrometry. *Journal of AOAC International*, *88*(4), pp.1118-1125.

Kim, S., Eichhorn, P., Jensen, J. N., Weber, A. S., Aga, D. S., 2005. Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environmental science & technology*, *39*(15), pp.5816-5823.

Kim, S., Eichhorn, P., Jensen, J. N., Weber, A. S., Aga, D. S., 2005. Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environmental science & technology*, *39*(15), pp.5816-5823.

Kim, S., Eichhorn, P., Jensen, J. N., Weber, A. S., Aga, D. S., 2005. Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environmental Science & Technology*, *39*(15), pp.5816-5823.

Kimosop, S.J., Getenga, Z.M., Orata, F., Okello, V.A., Cheruiyot, J.K., 2016. Residue levels and discharge loads of antibiotics in wastewater treatment plants (WWTPs), hospital lagoons, and rivers within Lake Victoria Basin, Kenya. *Environmental monitoring and assessment*, *188*(9), pp.532.

Knapp, C. W., Engemann, C. A., Hanson, M. L., Keen, P. L., Hall, K. J., Graham, D. W., 2008. Indirect evidence of transposon-mediated selection of antibiotic resistance genes in aquatic systems at low-level oxytetracycline exposures. *Environmental science & technology*, *42*(14), pp.5348-5353.

Kosma, C. I., Lambropoulou, D. A., & Albanis, T. A., 2010. Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece. *Journal of hazardous materials*, *179*(1-3), pp.804-817.

Kwon, J. W., 2016. Environmental impact assessment of veterinary drug on fish aquaculture for food safety. *Drug Testing and Analysis*, *8*(5-6), pp.556-564.

Lakshminarasimman, N., Quiñones, O., Vanderford, B. J., Campo-Moreno, P., Dickenson, E. V., McAvoy, D. C., 2018. Biotransformation and sorption of trace organic compounds in biological nutrient removal treatment systems. *Science of the Total Environment*, *640*, pp.62-72.

Lamm, A., Gozlan, I., Rotstein, A., Avisar, D., 2009. Detection of amoxicillin-diketopiperazine-2′, 5′ in wastewater samples. *Journal of Environmental Science and Health Part A*, *44*(14), pp.1512-1517.

Larcher, S., Yargeau, V., 2011. Biodegradation of sulfamethoxazole by individual and mixed bacteria. *Applied microbiology and biotechnology*, *91*(1), pp.211-218.

Larcher, S., Yargeau, V., 2011. Biodegradation of sulfamethoxazole by individual and mixed bacteria. *Applied Microbiology and Biotechnology*, *91*(1), pp.211-218.

Leng, Y., Bao, J., Chang, G., Zheng, H., Li, X., Du, J., Snow, D., Li, X., 2016. Biotransformation of tetracycline by a novel bacterial strain Stenotrophomonas maltophilia DT1. *Journal of Hazardous Materials*, *318*, pp.125-133.

Le Page, G., Gunnarsson, L., Snape, J., Tyler, C. R., 2017. Integrating human and environmental health in antibiotic risk assessment: a critical analysis of protection goals, species sensitivity and antimicrobial resistance. *Environment international*, *109*, pp.155-169.

Li, B., Zhang, T., 2010. Biodegradation and adsorption of antibiotics in the activated sludge process. *Environmental science & technology*, *44*(9), pp.3468-3473.

Li, B., Zhang, T., 2013. Removal mechanisms and kinetics of trace tetracycline by two types of activated sludge treating freshwater sewage and saline sewage. *Environmental Science and Pollution Research*, *20*(5), pp.3024-3033.

Li, R., Yuan, Q., Zhang, Y., Ling, J., Han, T., 2011. Hydrophilic interaction chromatographic determination of oxytetracycline in the environmental water using silica column. *Journal of liquid chromatography & related technologies*, *34*(7), pp.511-520.

Li, W., Shi, Y., Gao, L., Liu, J., Cai, Y., 2013. Occurrence, distribution and potential affecting factors of antibiotics in sewage sludge of wastewater treatment plants in China. *Science of The Total Environment*, *445*, pp.306-313.

Liu, Q., Xu, L., Ke, Y., Jin, Y., Zhang, F., Liang, X., 2011. Analysis of cephalosporins by hydrophilic interaction chromatography. *Journal of pharmaceutical and biomedical analysis*, *54*(3), pp.623-628.

Llorca, M., Rodríguez-Mozaz, S., Couillerot, O., Panigoni, K., de Gunzburg, J., Bayer, S., Czaja, R., Barceló, D., 2015. Identification of new transformation products during enzymatic treatment of tetracycline and erythromycin antibiotics at laboratory scale by an on-line turbulent flow liquid-chromatography coupled to a high resolution mass spectrometer LTQ-Orbitrap. *Chemosphere*, *119*, pp.90-98.

Lombardo-Agüí, M., Cruces-Blanco, C., García-Campaña, A.M., Gámiz-Gracia, L., 2014. Multiresidue analysis of quinolones in water by ultra-high perfomance liquid chromatography with tandem mass spectrometry using a simple and effective sample treatment. *Journal of separation science*, *37*(16), pp.2145-2152.

Längin, A., Alexy, R., König, A., Kümmerer, K., 2009. Deactivation and transformation products in biodegradability testing of ß-lactams amoxicillin and piperacillin. *Chemosphere*, *75*(3), pp.347-354.

López-Roldán, R., de Alda, M.L., Gros, M., Petrovic, M., Martín-Alonso, J., Barceló, D., 2010. Advanced monitoring of pharmaceuticals and estrogens in the Llobregat River basin (Spain) by liquid chromatography–triple quadrupole-tandem mass spectrometry in combination with ultra performance liquid chromatography–time of flight-mass spectrometry. *Chemosphere*, *80*(11), pp.1337-1344.

López-Serna, R., Pérez, S., Ginebreda, A., Petrović, M., Barceló, D., 2010. Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography-electrospray–tandem mass spectrometry. *Talanta*, *83*(2), pp.410-424.

Lützhøft, H.C.H., Halling-Sørensen, B., Jørgensen, S.E., 1999. Algal toxicity of antibacterial agents applied in Danish fish farming. *Archives of Environmental Contamination and Toxicology, 36*(1), pp.1-6.

Ma, C., Lo, P. K., Xu, J., Li, M., Jiang, Z., Li, G., Zhu, Q., Li, X., Leong, S. Y., Li, Q., 2020. Molecular mechanisms underlying lignocellulose degradation and antibiotic resistance genes removal revealed via metagenomics analysis during different agricultural wastes composting. *Bioresource Technology*, *314*, pp.123731.

Magee, H.Y., Maurer, M.M., Cobos, A., Pycke, B.F., Venkatesan, A.K., Magee, D., Scotch, M., Halden, R.U., 2018. US nationwide reconnaissance of ten infrequently monitored antibiotics in municipal biosolids. *Science of the Total Environment*, *643*, pp.460-467.

Majewsky, M., Glauner, T., Horn, H., 2015. Systematic suspect screening and identification of sulfonamide antibiotic transformation products in the aquatic environment. *Analytical and Bioanalytical Chemistry*, *407*(19), pp.5707-5717.

Mao, F., Liu, X., Wu, K., Zhou, C., Si, Y., 2018. Biodegradation of sulfonamides by Shewanella oneidensis MR-1 and Shewanella sp. strain MR-4. *Biodegradation*, *29*(2), pp.129-140.

Martinez, J.L., 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental pollution*, *157*(11), pp.2893-2902.

Massé, D. I., Saady, N. M. C., Gilbert, Y., 2014. Potential of biological processes to eliminate antibiotics in livestock manure: an overview. *Animals*, *4*(2), pp.146-163.

Mayers, D.L. ed., 2009. *Antimicrobial drug resistance* (pp. 803-824). New York: Humana press.

McArdell, C.S., Molnar, E., Suter, M.J.F., Giger, W., 2003. Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley Watershed, Switzerland. *Environmental science & technology*, *37*(24), pp.5479-5486.

McClure, E.L., Wong, C.S., 2007. Solid phase microextraction of macrolide, trimethoprim, and sulfonamide antibiotics in wastewaters. *Journal of Chromatography A*, *1169*(1-2), pp.53-62.

Miao, X.S., Bishay, F., Chen, M., Metcalfe, C.D., 2004. Occurrence of antimicrobials in the final effluents of wastewater treatment plants in Canada. *Environmental science & technology*, *38*(13), pp.3533-3541.

Michael, I., Rizzo, L., McArdell, C. S., Manaia, C. M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D., 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. *Water research*, *47*(3), pp.957-995.

Ministry of Health, Labour, and Welfare of Japan, 2019. Nippon AMR One Health Report (NAOR) 2019. https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000120172.html

Mitchell, S.M., Ullman, J.L., Teel, A.L., Watts, R.J., 2014. pH and temperature effects on the hydrolysis of three β-lactam antibiotics: Ampicillin, cefalotin and cefoxitin. *Science of the total environment*, *466*, pp.547-555.

Morehead, M. S., Scarbrough, C., 2018. Emergence of global antibiotic resistance. *Primary care: clinics in office practice*, *45*(3), pp.467-484.

Murata, A., Takada, H., Mutoh, K., Hosoda, H., Harada, A., Nakada, N., 2011. Nationwide monitoring of selected antibiotics: distribution and sources of sulfonamides, trimethoprim, and macrolides in Japanese rivers. *Science of the total Environment*, *409*(24), pp.5305-5312.

Narumiya, M., Nakada, N., Yamashita, N., Tanaka, H., 2013. Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. *Journal of hazardous materials*, *260*, pp.305-312.

Nguyen, L.N., Nghiem, L.D., Oh, S., 2018. Aerobic biotransformation of the antibiotic ciprofloxacin by Bradyrhizobium sp. isolated from activated sludge. *Chemosphere*, *211*, pp.600-607.

Nödler, K., Licha, T., Barbieri, M., Pérez, S., 2012. Evidence for the microbially mediated abiotic formation of reversible and non-reversible sulfamethoxazole transformation products during denitrification. *Water research*, *46*(7), pp.2131-2139.

Oberoi, A. S., Jia, Y., Zhang, H., Khanal, S. K., Lu, H., 2019. Insights into the fate and removal of antibiotics in engineered biological treatment systems: a critical review. *Environmental science & technology*, *53*(13), pp.7234-7264.

Oberoi, A. S., Jia, Y., Zhang, H., Khanal, S. K., Lu, H., 2019. Insights into the fate and removal of antibiotics in engineered biological treatment systems: a critical review. *Environmental science & technology*, *53*(13), pp.7234-7264.

Oka, H., Ito, Y., Matsumoto, H., 2000. Chromatographic analysis of tetracycline antibiotics in foods. *Journal of Chromatography A*, *882*(1-2), pp.109-133.

Okuda, T., Yamashita, N., Tanaka, H., Matsukawa, H., Tanabe, K., 2009. Development of extraction method of pharmaceuticals and their occurrences found in Japanese wastewater treatment plants. *Environment international*, *35*(5), pp.815-820.

Oosterhuis, M., Sacher, F., ter Laak, T.L., 2013. Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. *Science of the Total Environment*, *442*, pp.380-388.

Ort, C., Hollender, J., Schaerer, M., Siegrist, H., 2009. Model-based evaluation of reduction strategies for micropollutants from wastewater treatment plants in complex river networks. *Environmental Science & Technology*, *43*(9), pp.3214-3220.

Pailler, J.Y., Krein, A., Pfister, L., Hoffmann, L., Guignard, C., 2009. Solid phase extraction coupled to liquid chromatography-tandem mass spectrometry analysis of sulfonamides, tetracyclines, analgesics and hormones in surface water and wastewater in Luxembourg. *Science of the Total Environment*, *407*(16), pp.4736-4743.

Pallecchi, L., Bartoloni, A., Paradisi, F., Rossolini, G.M., 2008. Antibiotic resistance in the absence of antimicrobial use: mechanisms and implications. *Expert review of anti-infective therapy*, *6*(5), pp.725-732.

Park J., 2016. Removal characteristics and predictive model of pharmaceuticals and personal care products (PPCPs) in membrane bioreactor (MBR) process. Doctoral Thesis) Graduate School of Engineering, Kyoto University, Japan.

Pauwels, B., Verstraete, W., 2006. The treatment of hospital wastewater: an appraisal. *Journal of water and health*, *4*(4), pp.405-416.

Peng, J., Wang, X., Yin, F., Xu, G., 2019. Characterizing the removal routes of seven pharmaceuticals in the activated sludge process. *Science of The Total Environment*, *650*, pp.2437-2445.

Peng, X., Tan, J., Tang, C., Yu, Y., Wang, Z., 2008. Multiresidue determination of fluoroquinolone, sulfonamide, trimethoprim, and chloramphenicol antibiotics in urban waters in China. *Environmental Toxicology and Chemistry: An International Journal*, *27*(1), pp.73-79.

Peru, K.M., Kuchta, S.L., Headley, J.V., Cessna, A.J., 2006. Development of a hydrophilic interaction chromatography–mass spectrometry assay for spectinomycin and lincomycin in liquid hog manure supernatant and run-off from cropland. *Journal of Chromatography A*, *1107*(1-2), pp.152-158.

Petrovic, M., Farré, M., De Alda, M.L., Perez, S., Postigo, C., Köck, M., Radjenovic, J., Gros, M., Barcelo, D., 2010. Recent trends in the liquid chromatography–mass spectrometry analysis of organic contaminants in environmental samples. *Journal of Chromatography A*, *1217*(25), pp.4004-4017.

Picó, Y., Fernández, M., Ruiz, M.J., Font, G., 2007. Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment. *Journal of biochemical and biophysical methods*, *70*(2), pp.117-131.

Polesel, F., Andersen, H. R., Trapp, S., Plósz, B. G., 2016. Removal of antibiotics in biological wastewater treatment systems—a critical assessment using the activated sludge modeling framework for xenobiotics (ASM-X). *Environmental Science & Technology*, *50*(19), pp.10316-10334.

Polesel, F., Andersen, H.R., Trapp, S., Plósz, B.G., 2016. Removal of Antibiotics in Biological Wastewater Treatment Systems-A Critical Assessment Using the Activated Sludge Modeling Framework for Xenobiotics (ASM-X). *Environmental science & technology*, *50*(19), pp.10316-10334.

Pomies, M., Choubert, J. M., Wisniewski, C., Coquery, M., 2013. Modelling of micropollutant removal in biological wastewater treatments: a review. *Science of the Total Environment*, *443*, pp.733-748.

Pompei, C. M. E., Campos, L. C., da Silva, B. F., Fogo, J. C., & Vieira, E. M., 2019. Occurrence of PPCPs in a Brazilian water reservoir and their removal efficiency by ecological filtration. *Chemosphere*, *226*, pp.210-219.

Prieto, A., Möder, M., Rodil, R., Adrian, L., Marco-Urrea, E., 2011. Degradation of the antibiotics norfloxacin and ciprofloxacin by a white-rot fungus and identification of degradation products. *Bioresource Technology*, *102*(23), pp.10987-10995.

Primel, E. G., Caldas, S. S., Escarrone, A. L., 2012. Multi-residue analytical methods for the determination of pesticides and PPCPs in water by LC-MS/MS: a review. *Central European Journal of Chemistry*, *10*(3), pp.876-899.

Pérez-Lemus, N., López-Serna, R., Pérez-Elvira, S. I., Barrado, E., 2019. Analytical methodologies for the determination of pharmaceuticals and personal care products (PPCPs) in sewage sludge: A critical review. *Analytica chimica acta*, *1083*, pp.19-40.

Pérez-Parada, A., Agueera, A., Gómez-Ramos, M. D. M., García-Reyes, J. F., Heinzen, H., Fernández-Alba, A. R., 2011. Behavior of amoxicillin in wastewater and river water: identification of its main transformation products by liquid chromatography/electrospray quadrupole time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry*, *25*(6), pp.731-742.

Rao, R.N., Venkateswarlu, N., Narsimha, R., 2008. Determination of antibiotics in aquatic environment by solid-phase extraction followed by liquid chromatography–electrospray ionization mass spectrometry. *Journal of Chromatography A*, *1187*(1-2), pp.151-164.

Ricken, B., Kolvenbach, B.A., Bergesch, C., Benndorf, D., Kroll, K., Strnad, H., Vlček, Č., Adaixo, R., Hammes, F., Shahgaldian, P., Schäffer, A., 2017. FMNH 2-dependent monooxygenases initiate catabolism of sulfonamides in Microbacterium sp. strain BR1 subsisting on sulfonamide antibiotics. *Scientific reports*, *7*(1), pp.1-11.

Sacher, F., Lange, F.T., Brauch, H.J., Blankenhorn, I., 2001. Pharmaceuticals in groundwaters: analytical methods and results of a monitoring program in Baden-Württemberg, Germany. *Journal of chromatography A*, *938*(1-2), pp.199-210.

Sanderson, H., Brain, R. A., Johnson, D. J., Wilson, C. J., Solomon, K. R., 2004. Toxicity classification and evaluation of four pharmaceuticals classes: antibiotics, antineoplastics, cardiovascular, and sex hormones. *Toxicology*, *203*(1-3), pp.27-40.

Sanderson, H., Johnson, D. J., Wilson, C. J., Brain, R. A., Solomon, K. R., 2003. Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. *Toxicology letters*, *144*(3), pp.383-395.

Schiesel, S., Lämmerhofer, M., Lindner, W., 2010. Multitarget quantitative metabolic profiling of hydrophilic metabolites in fermentation broths of β-lactam antibiotics production by HILIC–ESI–MS/MS. *Analytical and bioanalytical chemistry*, *396*(5), pp.1655-1679.

Senta, I., Krizman-Matasic, I., Terzic, S., Ahel, M., 2017. Comprehensive determination of macrolide antibiotics, their synthesis intermediates and transformation products in wastewater effluents and ambient waters by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *1509*, pp.60-68.

Shi, Y. J., Wang, X. H., Qi, Z., Diao, M. H., Gao, M. M., Xing, S. F., Wang, S. G., Zhao, X. C., 2011. Sorption and biodegradation of tetracycline by nitrifying granules and the toxicity of tetracycline on granules. *Journal of Hazardous Materials*, *191*(1-3), pp.103-109.

Simazaki, D., Kubota, R., Suzuki, T., Akiba, M., Nishimura, T., Kunikane, S., 2015. Occurrence of selected pharmaceuticals at drinking water purification plants in Japan and implications for human health. *Water Research*, *76*, pp.187-200.

Stevens-Garmon, J., Drewes, J. E., Khan, S. J., McDonald, J. A., Dickenson, E. R., 2011. Sorption of emerging trace organic compounds onto wastewater sludge solids. *Water Research*, *45*(11), 3417-3426.

Stevens-Garmon, J., Drewes, J. E., Khan, S. J., McDonald, J. A., Dickenson, E. R.，2011. Sorption of emerging trace organic compounds onto wastewater sludge solids. *Water Research*, *45*(11), pp.3417-3426.

Stolker, A.A., Niesing, W., Hogendoorn, E.A., Versteegh, J.F., Fuchs, R., Udo, A.T., 2004. Liquid chromatography with triple-quadrupole or quadrupole-time of flight mass spectrometry for screening and confirmation of residues of pharmaceuticals in water. *Analytical and Bioanalytical Chemistry*, *378*(4), pp.955-963.

Suarez, S., Lema, J. M., Omil, F., 2010. Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. *Water Research*, *44*(10), pp.3214-3224.

Taboada-Santos, A., Behera, C. R., Sin, G., Gernaey, K. V., Mauricio-Iglesias, M., Carballa, M., Lema, J. M., 2020. Assessment of the fate of organic micropollutants in novel wastewater treatment plant configurations through an empirical mechanistic model. *Science of The Total Environment*, *716*, pp137079.

Taboada-Santos, A., Behera, C.R., Sin, G., Gernaey, K.V., Mauricio-Iglesias, M., Carballa, M., Lema, J.M., 2020. Assessment of the fate of organic micropollutants in novel wastewater treatment plant configurations through an empirical mechanistic model. *Science of the Total Environment*, *716*, p.137079.

Taboada-Santos, A., Braz, G. H., Fernandez-Gonzalez, N., Carballa, M., Lema, J. M., 2019. Thermal hydrolysis of sewage sludge partially removes organic micropollutants but does not enhance their anaerobic biotransformation. *Science of The Total Environment*, *690*, pp.534-542.

Takács, I., Patry, G. G., Nolasco, D., 1991. A dynamic model of the clarification-thickening process. *Water Research*, *25*(10), pp.1263-1271.

Tamtam, F., Mercier, F., Le Bot, B., Eurin, J., Dinh, Q.T., Clément, M., Chevreuil, M., 2008. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. *Science of the total environment*, *393*(1), pp.84-95.

Tamura, I., Yasuda, Y., Kagota, K. I., Yoneda, S., Nakada, N., Kumar, V., Kameda, Y., Kimura, K., Tatarazako, K., Yamamoto, H., 2017. Contribution of pharmaceuticals and personal care products (PPCPs) to whole toxicity of water samples collected in effluent-dominated urban streams. *Ecotoxicology and Environmental Safety*, *144*, pp.338-350.

Tanoue, R., Nomiyama, K., Nakamura, H., Kim, J.W., Isobe, T., Shinohara, R., Kunisue, T. and Tanabe, S., 2015. Uptake and tissue distribution of pharmaceuticals and personal care products in wild fish from treated-wastewater-impacted streams. *Environmental science & technology*, *49*(19), pp.11649-11658.

Tao, Y., Chen, D., Yu, H., Huang, L., Liu, Z., Cao, X., Yan, C., Pan, Y., Liu, Z., Yuan, Z., 2012. Simultaneous determination of 15 aminoglycoside (s) residues in animal derived foods by automated solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Food chemistry*, *135*(2), pp.676-683.

Taşkan, B., Hanay, Ö., Taşkan, E., Erdem, M., Hasar, H., 2016. Hydrogen-based membrane biofilm reactor for tetracycline removal: biodegradation, transformation products, and microbial community. *Environmental Science and Pollution Research*, *23*(21), pp.21703-21711.

Terzic, S., Senta, I., Matosic, M., Ahel, M., 2011. Identification of biotransformation products of macrolide and fluoroquinolone antimicrobials in membrane bioreactor treatment by ultrahigh-performance liquid chromatography/quadrupole time-of-flight mass spectrometry. *Analytical and Bioanalytical Chemistry*, *401*(1), pp.353-363.

Terzic, S., Udikovic-Kolic, N., Jurina, T., Krizman-Matasic, I., Senta, I., Mihaljevic, I., Loncar, J., Smital, T., Ahel, M., 2018. Biotransformation of macrolide antibiotics using enriched activated sludge culture: kinetics, transformation routes and ecotoxicological evaluation. *Journal of Hazardous Materials*, *349*, pp.143-152.

Torresi, E., Polesel, F., Bester, K., Christensson, M., Smets, B.F., Trapp, S., Andersen, H.R., Plósz, B.G., 2017. Diffusion and sorption of organic micropollutants in biofilms with varying thicknesses. *Water Research*, *123*, pp.388-400.

Tran, N.H., Chen, H., Van Do, T., Reinhard, M., Ngo, H.H., He, Y., Gin, K.Y.H., 2016. Simultaneous analysis of multiple classes of antimicrobials in environmental water samples using SPE coupled with UHPLC-ESI-MS/MS and isotope dilution. *Talanta*, *159*, pp.163-173.

Valcárcel, Y., Alonso, S.G., Rodríguez-Gil, J.L., Gil, A., Catalá, M., 2011. Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk. *Chemosphere*, *84*(10), pp.1336-1348.

Valette, J.C., Demesmay, C., Rocca, J.L., Verdon, E., 2004. Separation of tetracycline antibiotics by hydrophilic interaction chromatography using an amino-propyl stationary phase. *Chromatographia*, *59*(1-2), pp.55-60.

Van De Sande-Bruinsma, N., Grundmann, H., Verloo, D., Tiemersma, E., Monen, J., Goossens, H., Ferech, M. and European Antimicrobial Resistance Surveillance System, 2008. Antimicrobial drug use and resistance in Europe. *Emerging infectious diseases, 14*(11), pp.1722.

Vasudevan, D., Bruland, G.L., Torrance, B.S., Upchurch, V.G., MacKay, A.A., 2009. pH-dependent ciprofloxacin sorption to soils: interaction mechanisms and soil factors influencing sorption. *Geoderma*, *151*(3-4), pp.68-76.

Verlicchi, P., Al Aukidy, M., Jelic, A., Petrović, M., Barceló, D., 2014. Comparison of measured and predicted concentrations of selected pharmaceuticals in wastewater and surface water: a case study of a catchment area in the Po Valley (Italy). *Science of the Total Environment*, *470*, pp.844-854.

Verlicchi, P., Galletti, A., Petrovic, M., Barceló, D., Al Aukidy, M., Zambello, E., 2013. Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed—analysis of their respective contributions. *Science of the Total Environment*, *454*, pp.411-425.

Verlicchi, P., Zambello, E., 2016. Predicted and measured concentrations of pharmaceuticals in hospital effluents. Examination of the strengths and weaknesses of the two approaches through the analysis of a case study. *Science of the Total Environment*, *565*, pp.82-94.

Vieno, N.M., Tuhkanen, T., Kronberg, L., 2006. Analysis of neutral and basic pharmaceuticals in sewage treatment plants and in recipient rivers using solid phase extraction and liquid chromatography–tandem mass spectrometry detection. *Journal of Chromatography A*, *1134*(1-2), pp.101-111.

Volkers, G., Schuldt, L., Palm, G. J., Wright, G. D., Hinrichs, W., 2010. Crystallization and preliminary X-ray crystallographic analysis of the tetracycline-degrading monooxygenase TetX2 from Bacteroides thetaiotaomicron. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications*, *66*(5), pp.611-614.

Wagil, M., Maszkowska, J., Białk-Bielińska, A., Caban, M., Stepnowski, P., Kumirska, J., 2015. Determination of metronidazole residues in water, sediment and fish tissue samples. *Chemosphere*, *119*, pp.S28-S34.

Wang, L., Ben, W., Li, Y., Liu, C., Qiang, Z., 2018. Behavior of tetracycline and macrolide antibiotics in activated sludge process and their subsequent removal during sludge reduction by ozone. *Chemosphere*, *206*, pp.184-191.

Watkinson, A.J., Murby, E.J., Kolpin, D.W., Costanzo, S.D., 2009. The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. *Science of the total environment*, *407*(8), pp.2711-2723.

Wen, X., Jia, Y., Li, J., 2009. Degradation of tetracycline and oxytetracycline by crude lignin peroxidase prepared from Phanerochaete chrysosporium–a white rot fungus. *Chemosphere*, *75*(8), pp.1003-1007.

Wright, G. D., 2005. Bacterial resistance to antibiotics: enzymatic degradation and modification. *Advanced Drug Delivery Reviews*, *57*(10), pp.1451-1470.

Yang, L.H., Ying, G.G., Su, H.C., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth‐inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga pseudokirchneriella subcapitata. *Environmental Toxicology and Chemistry: An International Journal, 27*(5), pp.1201-1208.

Yang, S. F., Lin, C. F., Lin, A. Y. C., Hong, P. K. A., 2011. Sorption and biodegradation of sulfonamide antibiotics by activated sludge: experimental assessment using batch data obtained under aerobic conditions. *Water research*, *45*(11), pp.3389-3397.

Yang, S., Cha, J., Carlson, K., 2005. Simultaneous extraction and analysis of 11 tetracycline and sulfonamide antibiotics in influent and effluent domestic wastewater by solid-phase extraction and liquid chromatography-electrospray ionization tandem mass spectrometry. *Journal of Chromatography A*, *1097*(1-2), pp.40-53.

Ye, Z., Weinberg, H.S., Meyer, M.T., 2007. Trace analysis of trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry. *Analytical Chemistry*, *79*(3), pp.1135-1144.

Yu, C. P., Chu, K. H., 2009. Occurrence of pharmaceuticals and personal care products along the West Prong Little Pigeon River in east Tennessee, USA. *Chemosphere*, *75*(10), pp.1281-1286.

Yu, T. H., Lin, A. Y. C., Lateef, S. K., Lin, C. F., Yang, P. Y., 2009. Removal of antibiotics and non-steroidal anti-inflammatory drugs by extended sludge age biological process. *Chemosphere*, *77*(2), pp.175-181.

Zuccato, E., Castiglioni, S., Bagnati, R., Melis, M., Fanelli, R., 2010. Source, occurrence and fate of antibiotics in the Italian aquatic environment. *Journal of hazardous materials*, *179*(1-3), pp.1042-1048.

Chapter III

Predicted environmental concentrations (PECs) of antibiotics in the influent of sewage treatment plants (STPs) based on Japanese annual shipping/prescription data

3.1 Introduction

Over the last few decades, the occurrence of micropollutants like pharmaceuticals has been a topic of increasing concern worldwide due to their potential to cause undesirable ecological effects. Among pharmaceuticals, antibiotics have received particular attention since the correlations between antibiotic resistance and their total consumption and occurrence in environment had been studied from the last 90s (Asai et al., 2005; Bronzwaer et al., 2002; Van De Sande-Bruinsma et al., 2008). There are a number of routes for antibiotics entering into the aquatic environment, such as discharge of sewage treatment plants (STPs) effluent to surface and ground water, landfill leachate, sewer leakage or/and overflow, runoff from agricultural areas and so on (Carvalho and Santos, 2016; Heberer, 2002). For the human-used antibiotics, the discharge of treated wastewater from STPs is the main route, because the conventional wastewater treatment processes were mainly designed for the removal of nitrogen, phosphorus and organic matters, the removal of micropollutants were limited (Watkinson et al., 2007). For the further risk assessment and establishment of control policies, it is essential to monitor the discharge loads of antibiotics from the STPs.

Numerous articles have been published on the occurrences of antibiotics in STPs (Ghosh et al., 2016; Karthikeyan and Meyer, 2006; Xu et al., 2007). However, the occurrences of antibiotics exhibit great variability influenced by several factors, such as types, locations, seasons and so on. Moreover, monitoring of antibiotics is restricted by

the available analytical methods in laboratories, cost and time consumption. Therefore, estimation approach becomes a promising way.

Predicting the concentrations of antibiotics in the influents of STPs is very important for improving the accuracy on the discharging loads estimation from STPs. For human-used antibiotics, they cannot be completely metabolized in human bodies, it was reported that 30-90% of unchanged forms were excreted through urine and feces (Le-Minh et al., 2010). Therefore, there are several relevant factors should be considered in predicting model: consumption of human-used antibiotics, excretion rate of each antibiotic from human bodies, wastewater produced volume per inhabitant in studied aera. The consumption data significantly affect the accuracy of the predicting results. However, consumption data involve several sources of uncertainties such as regional bias, inappropriate use of a medication, household disposal through toilet, sales without prescription or illegal acquisition (Baz-Lomba et al., 2016). Therefore, it is essential to improve the accuracy by use different sources of data. In Japan, there are two available databases related to the human-used antibiotics consumption. One is Japanese annual shipping volumes of pharmaceuticals surveyed by Ministry of Health Labour and Welfare (MHLW), Japan, and the other is the National Database of Health Insurance Claims and Specific Health Checkups of Japan (NDB) which was also initiated by MHLW in 2009 to provide “big data” for electronic prescription-derived information on various health care services provided by the National Health Insurance in Japan. Japan consists of 47 prefectures, and the NDB provides not only the total number of prescriptions in Japan but a separate number for each of the 47 prefectures (Sato et al., 2018), which allowed us to investigate the regional diversity in prescription weights to modify the predicting results.

In this study, prediction model based on the consumption data calculated from both shipping volumes and prescription volumes was applied to obtain the predicted environmental concentrations (PECs) of influents in two target STPs located in different prefectures. The antibiotics that were highly consumed in Japan and have the available analytical methods in laboratories were chosen as targets, and monitoring data of the sewage influents flowing into two target STPs was conducted. The accuracies of the predicting model using two databases were evaluated by the measured environmental

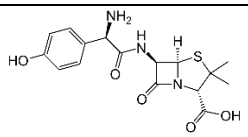
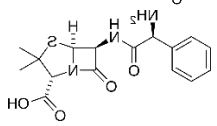
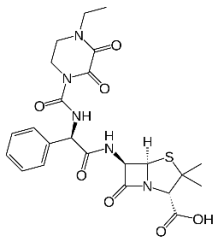
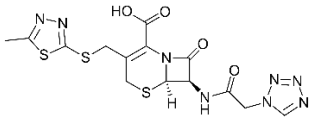
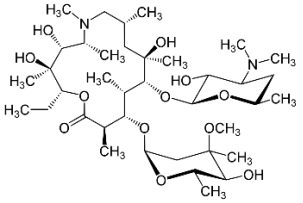
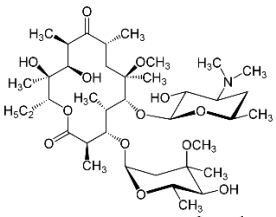
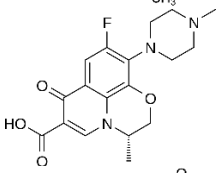
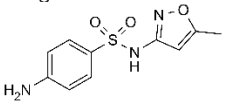
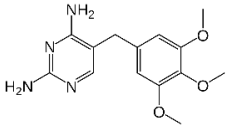
concentrations (MECs) of influents of two target STPs.

3.2 Materials and Methods

3.2.1 Chemicals and reagents

Amoxicillin trihydrate standard (purity 98%), ampicillin standard (purity 98%), cefazolin sodium salt standard (purity 98%), clarithromycin (purity 95%), levofloxacin (purity 98%), sulfamethoxazole (purity 99%) and trimethoprim (purity 99%) were purchased from Wako Pure Chemical Industry, Ltd. (Osaka, Japan). Azithromycin (purity 99%) was purchased from LKT Laboratories, Inc. Piperacillin (purity 95%) was purchased from the United States Pharmacopeial Convention, Inc (USP). Amoxicillin-d_4 (major), ampicillin-d_5 (Mixture of Diastereomers), azithromycin-d_3, caffeine-d_9, cefazolin-$^{13}C_2$,^{15}N sodium salt, clarithromycin-d_3, levofloxacin-d_8 and sulfamethoxazole-d_5 were purchased from Toronto Research Chemicals, Inc. LC-MS-grade solvents (methanol, acetonitrile), formic acid and ascorbic acid were purchased from Wako Pure Chemical Industry, Ltd. (Osaka, Japan). Individual standard and surrogate stock solutions of ampicillin and cefazolin sodium at 1 mg/ml were prepared in pure water: acetonitrile (75:25, v/v), ampicillin-d_5 and cefazolin-$^{13}C_2$,^{15}N sodium at 100 mg/L in pure water: acetonitrile (75:25, v/v), amoxicillin and piperacillin at 1 mg/ml in pure water: acetonitrile (50:50, v/v), and amoxicillin-d_4 at 100 mg/L in pure water: acetonitrile (50:50, v/v). Individual standard and surrogate stock solutions of azithromycin, clarithromycin, levofloxacin, sulfamethoxazole, trimethoprim, azithromycin-d_3, caffeine-d_9, clarithromycin-d_3, levofloxacin-d_8 and sulfamethoxazole-d_5 at 1 mg/ml were prepared in methanol. All the standard stock solutions were stored at -30 ℃. The properties of target antibiotics are shown in Table 3-1.

Table 3-1 Properties of target antibiotics

Compound	Formula	Molecular weight (g/mol)	Structure	Remarks
Amoxicillin	$C_{16}H_{19}N_3O_5S$	365.4		Penicillins, β-lactam antibiotic
Ampicillin	$C_{16}H_{19}N_3O_4S$	349.4		Penicillins, β-lactam antibiotic
Piperacillin	$C_{23}H_{27}N_5O_7S$	517.6		Penicillins, β-lactam antibiotic
Cefazolin	$C_{14}H_{14}N_8O_4S_3$	454.5		1st-generation cephalosporin, β-lactam antibiotic
Azithromycin	$C_{38}H_{72}N_2O_{12}$	749.0		Macrolide antibiotic
Clarithromycin	$C_{38}H_{69}NO_{13}$	748.0		Macrolide antibiotic
Levofloxacin	$C_{18}H_{20}FN_3O_4$	361.4		Quinolone antibiotic
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	253.3		Sulfonamide and trimethoprim antibiotic
Trimethoprim	$C_{14}H_{18}N_4O_3$	290.3		Sulfonamide and trimethoprim antibiotic

3.2.2 Sampling

Sewage influents were collected at two STPs (A and B), basic information of target STPs and investigation periods are given in Table 3-2. One-liter samples were collected in 1 L-glass bottles. All water samples were stored at 4 ℃ in the dark and processed within 24 h. Sampling was done on rain-free days, and no rainfall greater than 1 mm was observed in the 2 days before the sampling day.

Table 3-2 Basic information and investigation period of two target STPs

STP	Location	Main stream	Service population	Influent flow (m^3/d)	Investigation period
STP A	Prefecture S	Anaerobic/Anoxic/ Oxic	795,000	487,000	2019/05-2020/02 (n=6)
STP B	Prefecture F	Conventional activated sludge	340,000	171,935	2019/10-2020/03 (n=6)

3.2.3 Analytical procedure

For β-lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), we only determined the concentrations in liquid phase due to the determining limitations. For the other antibiotics, the concentrations in both liquid and solid phases were measured. Twenty-ml portions of each sample were filtered through the glass fiber filter (Whatman GF/B, 1 μm) for the analysis of β-lactam antibiotics. Ethylene diamine tetraacetic acid disodium (EDTA-2Na) of 1 g/L and a mixture of surrogate standard were added after filtration, and the samples were adjusted to pH 3 with 1 N HCl. The extraction process was performed by solid phase extraction (SPE) with Oasis HLB (200mg, 6cc, Waters), in which the cartridges were conditioned by washing with 3 mL of methanol and 3 mL of Milli-Q water preadjusted to pH 3, and the samples were transferred to the cartridges at a flow rate of 5 mL/min. After drying of the SPE cartridges by using a vacuum pump for 2 h, elution was performed with 6 mL of methanol. The elution solvent was evaporated to dryness by a gentle stream of nitrogen gas and redissolved in 1 mL of Milli-Q water. For the other antibiotics, a 100-ml sample was also filtered through a 1-μm glass fiber filter, the filtrate was defined as the liquid phase and residue at the filter as the solid phase. Each phase was spiked with a mixture of surrogate standards. The pretreatment procedures of the liquid phase were similar to

the procedure of β-lactam antibiotics, except 1 mL of an 85:15 (v/v) mixture of 0.1% formic acid and methanol was used as the redissolved solution. To extract the solid-phase samples, we prepared water at three pH level (pH 7, pH 2 by 1 N HCl, and pH 11 by 2M NaOH) and mixed in methanol at a 9:1 (v/v) ratio (Narumiya et al., 2013). The solid phase was extracted twice at pH 7, once at pH 2 and twice at pH 11, ultrasonication (As one, ASU-20D) and centrifuge (2500 rmp, Kubota, Centrifuge 4000) were repetitively used to collect supernatant of solid samples. And then the supernatant was filtered through the 1-μm glass fiber filter and diluted to 200 mL by Milli-Q water, which would be pretreated following liquid-phase procedures after that.

Ten μL of the 1 mL final extract was individually subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis with a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) device equipped with an ACQUITY BEH C_{18} (octadecylsilica-based) column (1.7 μm, 2.1 mm × 100 mm, Waters) (Narumiya et al., 2013). A gradient elution program was achieved at 60℃ with a mixed solvent system of 0.1% formic acid (v/v) in Milli-Q water (A) and methanol (B) at a flow rate of 0.35 mL/min under a program of 0.0-7.0 min (10% B), 7.0-7.1 (20% B), 7.1-8.0 (20% B), 8.0-12.0 (50% B), 12.0-16.0 (50% B), 16.0-16.1 (60% B), 16.1-20.0 (70% B), 20.0-21.0 (95% B), 21.0-23.0 (10% B) to condition the column. Relative optimum LC-MS/MS analyzing conditions of target compounds are shown in Table 3-3.

Table 3-3 Optimum LC-MS/MS conditions for the analysis of target antibiotics

Target compound	Native					Surrogate or representative surrogate					
	RT (min)	Precursor ion (m/z)	Product ion (m/z)	CV (V)	CE (eV)	RT (min)	Ion	Precursor ion (m/z)	Product ion (m/z)	CV (V)	CE (eV)
Amoxicillin	1.61	366.2	208.1/349.2[a]	18/20	14/10	1.59	+	371.2	160.2/354.5[a]	18/20	15/10
Trimethoprim	4.95	291.0	123.1[a]/230.1	42/42	26/24	4.76	+	204.0	144.0[a]/116.0	38/38	20/24
Levofloxacin	6.24	362.2	58.1[a]/261.1	38/38	36/28	6.05	+	370.3	62.3[a]/265.2	40/40	30/30
Sulfamethoxazole	7.45	254.0	92.1[a]/156.0	30/30	30/16	7.26	+	258	96.1/160.0	30/30	26/18
Ampicillin	8.65	351.3	106.3[a]/174.9	15/22	18/15	8.61	+	355.0	197.2	18	18
Cefazolin	9.00	455.2	156.0/323.1[a]	20/18	15/12	9.00	+	457.8	298.1/325.9[a]	20/20	15/13
Azithromycin	11.59	375.2	83.0[a]/116.0	24/24	26/26	11.59	+	376.8	83.0/158.1[a]	26/26	26/30
Piperacillin*	13.35	518.0	143.1[a]/160.0	20/18	18/12	8.61	+	355.0	197.2	18	18
Clarithromycin	15.34	748.6	83.0/158.1[a]	36/36	52/30	15.34	+	751.7	83.0/161.1[a]	38/38	54/32

All the analytes were analyzed in positive ionization mode. *: ampicillin-d_5 as surrogate

RT: retention time; CV: Cone Voltage; CE: Collision Energy; a: Quantitation ion.

3.2.4 Human-used antibiotic consumption

There are two public databases which are related to the human-used antibiotic consumption in Japan. One is the annual special drugs shipping database surveyed by MHLW which provides the annual shipping volumes of human-used antibiotics. The other is the National Database of Health Insurance Claims and Specific Health Checkups of Japan (NDB) which was also initiated by MHLW to provide electronic prescription-derived information on various health care services provided by the National Health Insurance in Japan. The NDB covers around 98% of the prescription provided by health insurance (Fujimori, 2016). In addition, the NDB not only provides the annual national volumes of human-used antibiotics, but also provides the annual volumes of each prefecture. To evaluate the regional bias, the data of Prefecture S and F were also collected. The per capita annual consumption [*I*, mg/ (inhabitant· year)] of each antibiotic was calculated by annual shipping/prescription volumes (*C*, mg/year) and population in Japan/ corresponding prefectures (*P*, inhabitant) [Equation 3-1]:

$$I = \frac{C}{P} \qquad \text{(Equation 3-1)}$$

According to the Statistical Handbook of Japan from 2014-2016 (Statistics Bureau, Japan), the population in Japan is 127,083,000, 127,095,000 and 126,933,000 inhabitants, respectively. In Prefecture S, the population is 1,416,000, 1,413,000 and 1,413,000 inhabitants, respectively, and corresponding 5,091,000, 5,102,000 and 5,104,000 in Prefecture F, respectively.

The latest data released in the databases is the data three years ago, and we collected the data of the last three years released by the databases. To improve the accuracy of estimation, the values used in the prediction model according to the following principles: if the volumes of specific antibiotic increased or decreased by year, the volume of the latest year would be used; if there is no obvious trend by year, the average value of the latest three years would be used.

3.2.5 Predicted environmental concentrations of sewage influent

The per capita annual consumption volumes [*I*, mg/ (inhabitant· year)] calculated by

the national shipping/prescription volumes and regional prescription volumes, the service population of each STP (p, inhabitant), the average flow rate of target STPs (Q, m^3/d) and excretion rate of unchanged compound of each antibiotic from human bodies (e, %, Table 3-4) were applied to obtain the predicted environmental concentration of STPs influent (PEC_{inf}, ng/L) by Equation 3-2 (Carballa et al., 2008):

$$PEC_{inf}=\frac{I\times p\times e}{Q\times 365}\times 1000 \qquad \text{(Equation 3-2)}$$

In addition, a range values of PEC_{inf} would be obtained correspondingly due to the range values of excretion rates. The individual antibiotics with the per capita annual consumption volumes higher than 20 mg/ (inhabitant· year) were chosen as the target compounds for the prediction.

Table 3-4 Excretion rates of selected antibiotics

Class	Compound	Excretion rate (%)		Reference
		min	max	
β-lactam-Penicillins	Ampicillin	30	40	Neu, 1974
	Piperacillin	45	55	Hayashi et al., 2010
	Amoxicillin	50	70	Neu, 1974
Other β-lactam-Cephalosporins	Cefazolin	75	90	Rattie and Ravin, 1975
	Cephalexin	90	90	Spyker et al., 1978
	Cefotiam	50	67	Brisson et al., 2984
	Cefaclor	62	80	Spyker et al., 1978
	Cefmetazole	62	75	Ko et al., 1989
	Ceftriaxone	33	53	Patel et al., 1981
	Cefdinir	10	20	Yilmaz and Paterson, 2010
	Cefcapene	33	41	Totsuka et al., 1992
	Cefditoren	18	20	Balbisi, 2002
	Meropenem	65	70	Harrison et al., 1989
Macrolides	Clarithromycin	18	40	Ferrero et al, 1990
	Azithromycin	6	20	Foulds et al., 1990
Quinolones	Tosufloxacin	26	45	Dauphin et al., 1993
	Levofloxacin	70	80	Riva et al., 2015
Sulfonamides & Trimethoprim	Sulfasalazine	15	15	Schröder and Campbell, 1972
	Sulfamethoxazole	15	20	Pérez et al., 2005
	Trimethoprim	43	60	Pérez et al., 2005

3.2.6 Comparison of measured influent concentration and PEC_{inf}

To evaluate the accuracy of the predicted equation, a comparison of predicted and measured concentrations of investigated compounds in two STPs is calculated by the ratio PEC_{inf} and the average measured concentration (MEC_{inf_avg}). The accuracy evaluation criteria proposed in a previous literature were applied (Ort et al., 2009; Verlicchi et al., 2014):

If $0.5 < PEC_{inf}/MEC_{inf\_avg} < 2$, then the PEC_{inf} is acceptable;

If $PEC_{inf}/MEC_{inf\_avg} < 0.5$, then the PEC_{inf} is unacceptably low;

If $PEC_{inf}/MEC_{inf\_avg} > 2$, then the PEC_{inf} is unacceptably high.

3.3 Results and Discussions

3.3.1 Measured environmental concentrations of sewage influents (MEC_{inf})

Nine target antibiotics, which were highly used in Japan and have available analytical methods, were monitored in influent of two STPs. It has been investigated for 6 times between May, 2019 and March, 2020, and the results are shown in Figure 3-1. In STP A, the concentrations of target antibiotics in influents were ranged from 28 ng/L (ampicillin) to 1235 ng/L (levofloxacin), while in STP B, it was ranged from 35 ng/L (cefazolin) to 1471 ng/L (levofloxacin). Among the target antibiotics, levofloxacin occurred at the highest concentration in the influent of both STP A (363-1235 ng/L) and STP B (547-1471 ng/L), followed by clarithromycin (337-749 ng/L), sulfamethoxazole (99-774 ng/L), piperacillin (85-355 ng/L), azithromycin (160-215 ng/L), trimethoprim (92-199 ng/L), amoxicillin (65-104 ng/L), cefazolin (50-105 ng/L), and ampicillin (28-85 ng/L) in STP A, and followed by clarithromycin (355-756 ng/L), piperacillin (213-875 ng/L), sulfamethoxazole (138-525 ng/L), cefazolin (35-453 ng/L), amoxicillin (103-405 ng/L), azithromycin (117-306 ng/L), ampicillin (60-357 ng/L) and trimethoprim (100-175 ng/L) in STP B. Except for sulfamethoxazole and trimethoprim, the ranges of $MECs_{inf}$ of each target antibiotic in STP B were higher than those in STP A. It indicates that the seasonal fluctuations in STP B were relatively larger than that of STP A.

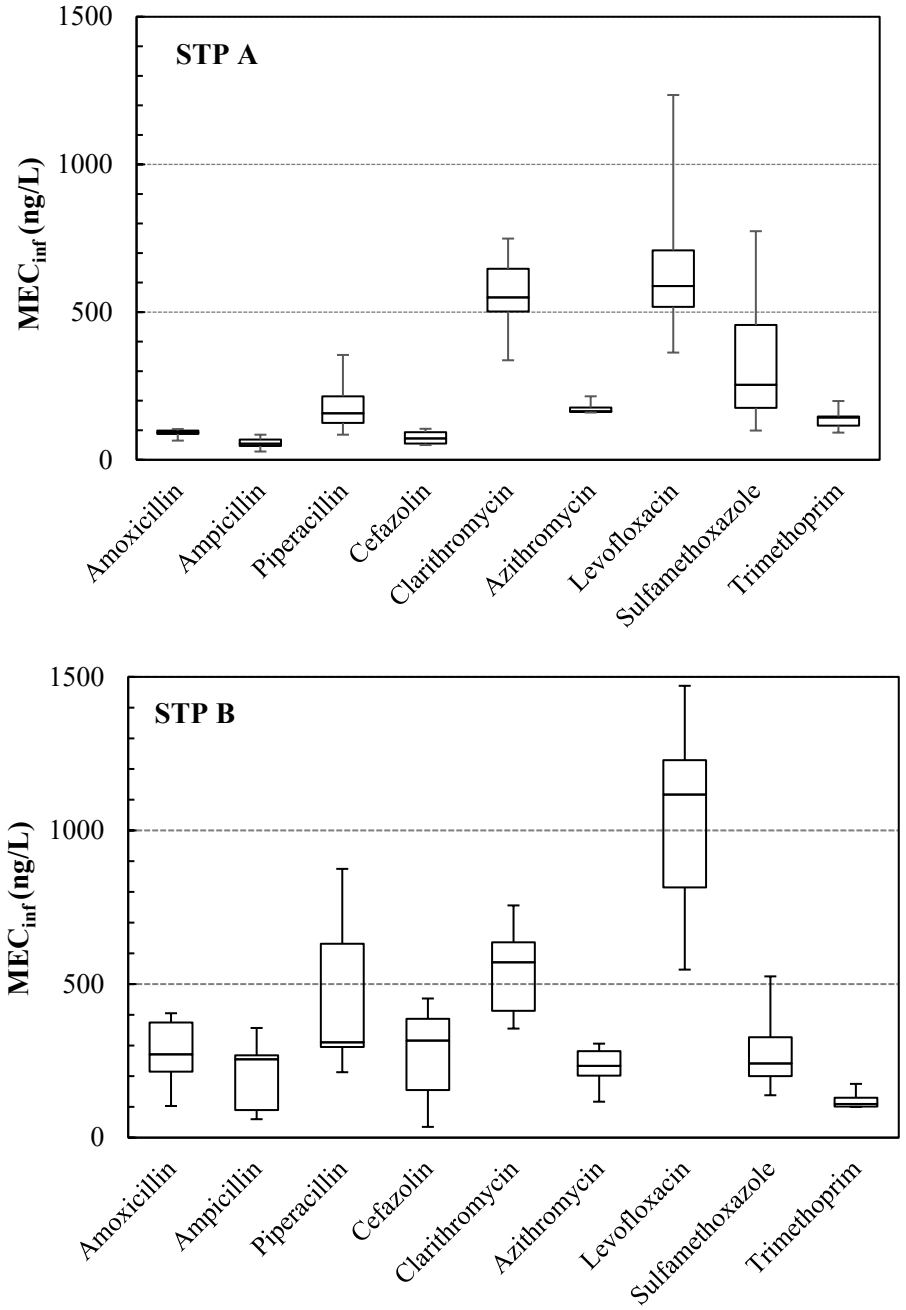


Figure 3-1 Measured concentrations of target antibiotics in sewage influents from STP A and STP B.

There are few investigations on the β-lactam antibiotics concentration in municipal sewage influent, while for other target antibiotics, the concentrations in sewage influent were in agreement with the results in previous studies conducted in Japan, such as levofloxacin (532-425 ng/L, Ghosh et al., 2016), clarithromycin (886-1688 ng/L, Azuma et al., 2015; 1129 ng/L, Ghosh et al., 2016), azithromycin (260 ng/L, Yasojima

et al., 2006), and sulfamethoxazole (159-174 ng/L, Ghosh et al., 2016). In European countries, the concentration ranges of amoxicillin, ampicillin and piperacillin were 18-6196 ng/L in sewage influents (Opriş et al., 2013; Carvalho and Santos, 2016), which were higher than those in our study. Sulfamethoxazole and trimethoprim are often used together and were found in similar concentrations to this study in sewage influent in Europe (Birošová et al., 2014) and in higher concentrations in China (>1578 ng/L) (Ghosh et al., 2016). In Europe, clarithromycin (694-2700 ng/L) and azithromycin (240-1490 ng/L) were detected in a little higher concentration compared to Japan, and levofloxacin concentrations (48-184 ng/L) were lower in sewage influents (Birošová et al., 2014).

3.3.2 Predicted environmental concentrations of sewage influent

The individual human-used antibiotic consumption volumes from 2014 to 2016 calculated by national shipping and prescription volumes were summarized in Table 3-5. The patterns of consumption of human-used antibiotics changed over the years. The shipping volumes of antibiotics are generally higher than prescription volumes. According to the results, consumption of amoxicillin, piperacillin, clarithromycin, azithromycin, levofloxacin, and Tosufloxacin calculated by national shipping volumes fluctuated seriously with year. It might be caused by the statistical errors of the original database, or these antibiotics were shipped and stocked by some special reasons. As a result, the patterns of the total volume of human-used antibiotics calculated by shipping data might not represent the real consumption variations in Japan. Compared to 2014, the total prescription volumes of human-used antibiotics increased by 17% in 2015, from 424.93 tons to 495.75 tons; while it decreased to 492.76 tons in 2016. The amount of β-lactam antibiotics prescribed in Japan are the highest, which accounting for almost half of total amount of antibiotics. Among the class of β-lactam antibiotics, the prescription amounts of penicillins, which contain amoxicillin, ampicillin and piperacillin, represent for around 24% of total amounts of human-used antibiotics. In Germany, the prescribed β-lactam antibiotics represented 73% of total amount, and penicillins (mostly amoxicillin and penicillin V) accounted for 53%, which were higher than in Japan (Kümmerer and Henninger, 2003). The prescription volumes of

macrolides, aminoglycosides and quinolones account for around 20%, 13% and 10%, representatively. In addition, the combination of sulfamethoxazole and trimethoprim represents for 5% of the total amount of antibiotics.

Table 3-5 Human-used antibiotics consumption data from 2014 to 2016 calculated by shipping and prescription volumes in Japan

Type		Compound	Shipping volume (t/year)			Prescription volume (t/year)		
			2014	2015	2016	2014	2015	2016
β-Lactams	Penicillins	Amoxicillin*	139.52	46.16	32.09	49.62	55.63	54.05
		Ampicillin	18.79	22.53	23.45	16.87	16.09	17.90
		Piperacillin*	7.17	85.25	644.55	35.72	42.39	44.50
	1-generation cephalosporin	Cefazolin	19.91	20.46	21.83	10.39	14.72	14.83
		Cephalexin	0.00	2.93	3.19	0.07	2.19	2.48
	2-generation cephalosporin	Cefaclor	16.49	10.49	10.45	2.71	5.10	5.34
		Cefmetazole	8.87	6.89	7.38	3.23	5.58	5.90
		Cefotiam	5.24	4.57	4.42	2.56	4.66	3.79
	3-generation cephalosporin	Cefcapene	38.49	31.57	32.32	25.81	27.55	25.78
		Cefditoren	24.00	24.52	19.96	19.15	20.80	19.55
		Ceftriaxone	17.16	20.06	20.63	9.04	13.82	14.48
		Cefdinir	11.13	10.75	9.66	6.85	8.52	7.99
		Cefoperazone	4.03	2.69	2.94	1.49	2.76	2.56
		Cefteram	3.78	0.62	0.59	1.69	2.18	1.82
		Cefpodoxime	2.57	2.44	2.38	1.34	2.27	2.14
		Ceftazidime	2.25	2.03	1.82	0.00	1.30	1.22
		Cefixime	0.00	0.00	0.00	0.00	0.19	0.17
	4-generation cephalosporin	Cefepime	3.90	3.88	3.74	1.87	2.72	2.98
	Carbapenems	Meropenem	6.24	6.57	7.23	4.45	5.70	5.91
		Imipenem	0.74	0.73	0.21	0.00	0.46	0.39
Macrolides		Clarithromycin*	168.23	107.98	104.79	71.96	89.69	85.32
		Azithromycin*	193.10	32.28	11.49	4.05	8.25	8.19
		Roxithromycin	6.87	7.37	7.30	3.43	5.84	5.61
Lincomycin		Clindamycin	2.44	2.59	2.71	2.01	2.01	1.94
Tetracyclines		Minocycline	3.47	3.50	3.59	4.88	4.95	4.95
		Doxycycline	0.00	0.00	0.00	0.89	0.93	1.05
Aminoglycoside		Sulfasalazine	62.44	64.21	69.75	60.44	62.44	63.93
		Amikacin	0.41	0.46	0.34	0.19	0.22	0.22
		Arbekacin	0.04	0.04	0.03	0.06	0.02	0.02
		Isepamicin	0.35	0.42	0.31	0.39	0.38	0.34
		Gentamicin	0.00	0.07	0.00	0.00	0.01	0.01
Quinolone		Levofloxacin*	410.83	212.03	43.75	43.54	36.86	36.35

Quinolone	Tosufloxacin*	25.79	5.90	7.46	4.66	5.97	5.94
	Ciprofloxacin	3.19	3.05	2.28	1.40	2.12	2.06
	Ofloxacin	1.27	0.96	0.88	0.35	0.58	0.58
	Norfloxacin	0.00	0.00	0.00	0.52	0.96	0.90
Glycopeptides	Fosfomycin	6.53	5.91	6.12	9.35	13.43	13.20
	Vancomycin	2.35	2.05	2.76	2.00	2.07	2.06
	Teicoplanin	0.14	0.12	0.12	0.06	0.10	0.10
Sulfonamides and trimethoprim	Sulfamethoxazole	22.77	21.13	-	18.26	20.24	21.76
	Trimethoprim	4.55	4.23	-	3.65	4.05	4.35

*: shipping volume fluctuated seriously with year; -: no data

After calculation by population and selection following the principles above, the *I* values of individual antibiotics calculated by national shipping/prescription data and regional prescription data of Prefecture S and F are given in Figure 3-2. Except azithromycin, the gap of *I* values calculated by national shipping and prescription data became less after selection by the strategies in 3.2.4. The sum of the *I* value of all human-used antibiotics calculated by the prescription of nation, Prefecture S and Prefecture F are 3708.40, 3363.81 and 3439.11 mg/(inhabitant·year), respectively. According to the age distribution in Japan in 2015 (Statistics Bureau, Japan), the population over 65 years old accounts for 26.6% in Japan, while these proportions are 24.2% and 25.9% in Prefecture S and F, respectively. Since aged people are more susceptible to infectious diseases (Gavazzi and Krause, 2002), the proportion of aged people (over 65) in Prefecture S and F are lower than the national level, which might result in the less usage of antibiotics. Compared the individual national prescribed antibiotics data and the regional prescribed data (Prefecture S and F), the regional consumption bias varied by individual antibiotics. The per capita annual consumptions of ampicillin, cefcapene, meropenem, azithromycin, and the combination of sulfamethoxazole and trimethoprim in Prefecture S were higher than the national average level, for the other antibiotics, the per capita annual consumptions were lower than the national average level. In Prefecture F, the per capita annual consumption of cefazolin, cefditoren, ceftriaxone, cefdinir, meropenem, azithromycin, levofloxacin and tosufloxacin were higher than the national average level.

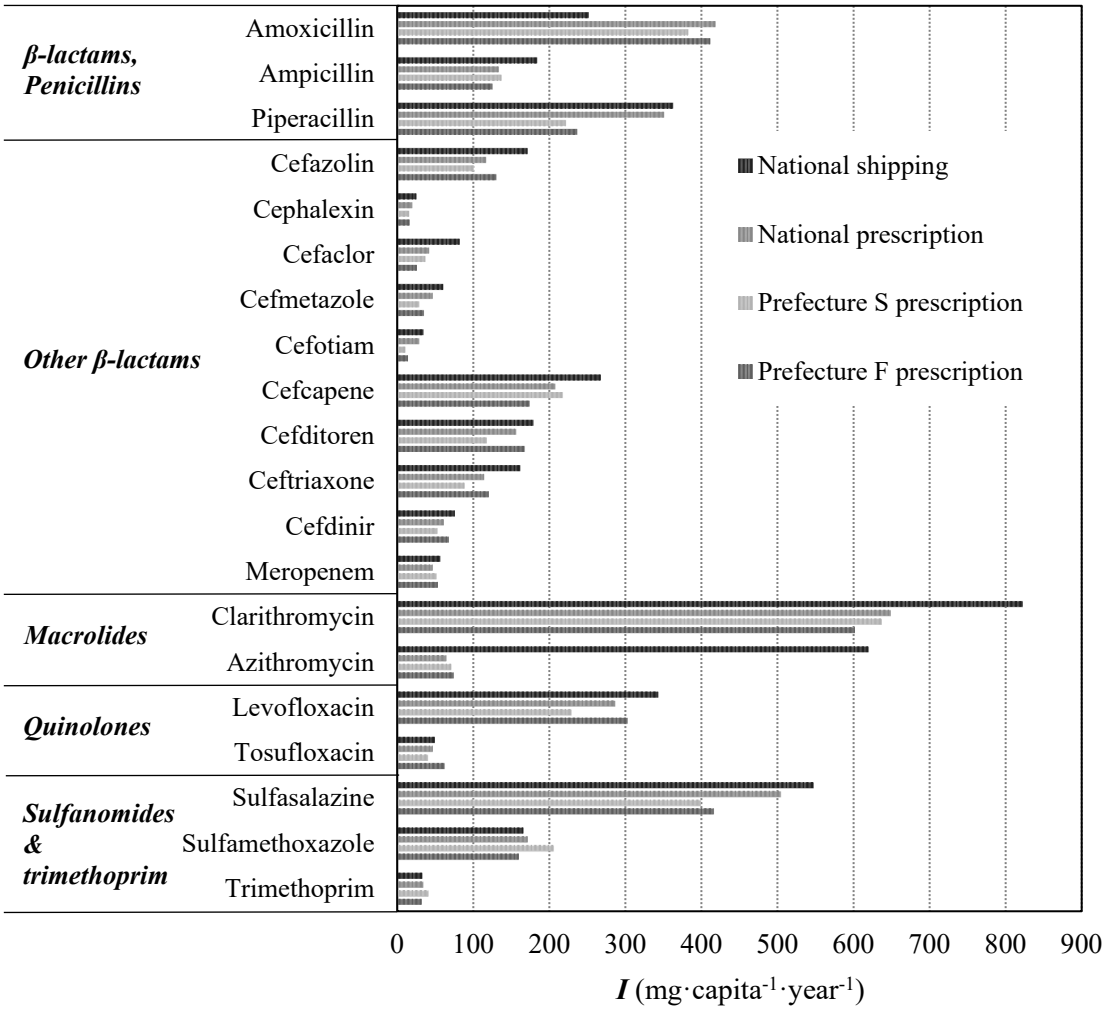


Figure 3-2 Individual human-used antibiotic consumption calculated by national shipping, national prescription and regional prescription data in Japan, respectively, expressed as per capita annual consumption (I) ($I_{individual}$>20 mg·capita^{-1}·year^{-1}were shown).

The predicted concentrations of selected antibiotics in sewage influent based on the national shipping, national prescription and regional prescription data are summarized in Table 3-6, the range of predicted concentrations came from the range of excretion rate of each antibiotic. The PEC_{inf} of STP A were slightly lower than those of STP B based from the same data source, which is directly related to the per capita sewage production, 613 and 506 L/(capita·d), respectively. The predicted concentrations based on the national shipping data were slightly higher than those predicted by using national prescription data in the case of most antibiotics. In contrast, the predicted concentrations of amoxicillin on the basis of national prescription data tended to be about two times

those predicted by national shipping data; for azithromycin, the concentrations predicted by national shipping volumes were about 10 times those of national prescription data, which might be caused by statistical error of original database.

In STP A, the predicted concentrations on the basis of national prescription volumes generally agreed with those on the basis of prescription data of Prefecture S, except for piperacillin and sulfasalazine. The PEC_{inf} of piperacillin and sulfasalazine based on the prescription volumes in Prefecture S were much lower than those predicted by the national prescription volumes, which resulted from the regional bias of antibiotics consumption. In STP B, similar to STP A, the PEC_{inf} of piperacillin and cefotiam on the basis of prescription volumes in Prefecture F were much lower than those of national prescription volumes. In the two STPs, the highest predicted concentrations were estimated for levofloxacin and clarithromycin, with a range of concentrations between 512 and 1780 ng/L, followed by amoxicillin (563-1585 ng/L) and piperacillin (446-1080 ng/L). Azuma et al. (2015) applied the shipping volume and sales volume of antibiotics and obtained the PEC_{inf} for clarithromycin with a range between 653 and 1923 ng/L in three Japanese STPs, which were similar to our results. The highest predicted concentration were estimated for clarithromycin (3760 ng/L) in an Italian STP (Verlicchi et al., 2014). Kümmerer and Henninger (2003) predicted the concentrations of antibiotics in sewage influent based on the annual consumption volumes in Germany and obtained a variety of different results. For example, PEC_{inf} of amoxicillin (257000 ng/L), piperacillin (2210 ng/L) and cefazolin (2060 ng/L) were much higher than those in our study, while PEC_{inf} of clarithromycin (540 ng/L), azithromycin (110 ng/L) and levofloxacin (140 ng/L) were lower than those in our study. The differences with the results from other countries were resulted from the different patterns of antibiotic consumption, and the differences of per capita sewage production would also be an important factor.

Table 3-6 Predicted concentrations of target antibiotics in sewage influent (the range of the results are caused by the excretion rate)

Compound	**PEC_{inf} (ng/L)**					
	National shipping data		National prescription data		Prefecture S prescription data	Prefecture F prescription data
	STP A	STP B	STP A	STP B	STP A	STP B
Amoxicillin	563-788	682-954	935-1309	1132-1585	855-1197	1115-1561
Ampicillin	247-329	299-398	179-239	217-289	184-245	203-271
Piperacillin	729-891	883-1080	706-862	855-1045	446-545	577-705
Cefazolin	574-689	695-834	392-470	475-570	337-404	529-635
Cephalexin*	101	122	79	95	63	79
Cefaclor*	227-293	275-355	117-150	141-182	102-132	86-111
Cefmetazole*	168-203	203-246	129-156	156-189	80-97	119-143
Cefotiam*	77-104	94-126	65-87	78-105	24-33	38-51
Cefcapene*	395-491	478-594	306-381	371-461	321-398	311-387
Cefditoren*	144-160	175-194	126-140	152-169	95-105	163-182
Ceftriaxone*	239-383	289-464	168-270	204-328	131-210	215-345
Cefdinir*	34-68	41-82	27-55	33-66	24-47	37-74
Meropenem*	165-177	199-215	135-146	164-177	150-162	187-202
Clarithromycin	661-1470	801-1780	522-1159	632-1404	512-1138	587-1304
Azithromycin	166-554	201-671	18-58	21-70	19-64	24-81
Sulfasalazine*	367	444	338	409	267	338
Levofloxacin	1074-1227	1301-1487	897-1025	1086-1241	716-818	1149-1313
Tosufloxacin*	57-100	70-121	54-94	66-114	47-81	88-152
Sulfamethoxazole	111-148	135-180	115-153	139-186	138-184	130-173
Trimethoprim	64-89	77-108	66-92	80-111	79-110	74-104

*: Not directly analyzed in this study.

3.3.3 Comparison of MEC_{inf} and PEC_{inf}

A comparison of predicted and measured concentrations of target antibiotics in the two STPs were carried out by the ratio between the predicted concentrations (on the basis of national shipping/prescription and regional prescription volumes) and the average value of measured concentrations (PEC_{inf}/MEC_{inf_avg}) (Figure 3-3), to evaluate the accuracy of the predicted equation.

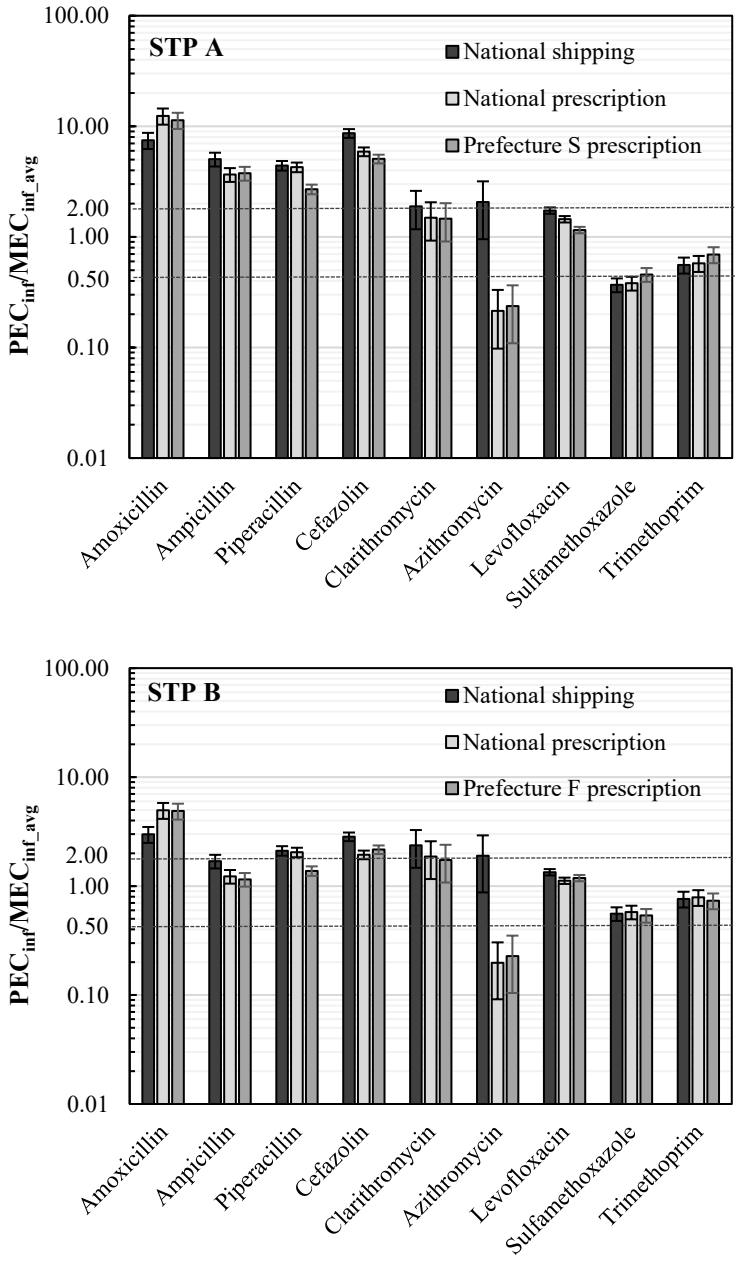


Figure 3-3 Comparison of predicted and measured concentrations of selected antibiotics in sewage influents, the height of the histogram represents the average values of PEC_{inf}/MEC_{inf_avg}, error bar represents the range of PEC_{inf}/MEC_{inf_avg}.

In two STPs, among the 9 detected antibiotics, 7 have a $PECs_{inf}$ calculated by national shipping volume greater than the corresponding $MECs_{inf}$, and 6 have a $PECs_{inf}$ calculated based on national/regional prescription volume greater than the corresponding $MECs_{inf}$. For β-lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), $PECs_{inf}$ based on three data sources were all unacceptably high

($PEC_{inf}/MEC_{inf\_avg}>2$) in STP A. In STP B, $PECs_{inf}$ of β-lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin) are greater than the corresponding $MECs_{inf}$. For amoxicillin, the predicted concentrations on the basis of three data sources are overestimated ($PEC_{inf}/MEC_{inf\_avg}>2$). The predicted concentrations of ampicillin are acceptable, and the $PECs_{inf}$ calculated by the regional prescription volumes are more closed to $MECs_{inf}$ (PEC_{inf}/MEC_{inf_avg}: 0.99-1.32). The $PECs_{inf}$ of piperacillin on the basis of national shipping (PEC_{inf}/MEC_{inf_avg}: 1.90-2.32) and prescription volumes (PEC_{inf}/MEC_{inf_avg}: 1.84-2.25) are overestimated, while it is acceptable that calculated by the regional prescription volume (PEC_{inf}/MEC_{inf_avg}: 1.24-1.52). For cefazolin, the PEC_{inf}/MEC_{inf_avg} on the basis of national shipping volume (2.58-3.10), national prescription volume (1.76-2.12) and regional prescription volume (1.97-2.36) are around 2 or higher than 2, which means the overestimation of predicted concentration. Additionally, the $PECs_{inf}$ on the basis of prescription volumes are closer to $MECs_{inf}$ than those on the basis of shipping volume. The discrepancies found between $PECs_{inf}$ and $MECs_{inf}$ for β-lactam antibiotics could be attribute to the unstability of β-lactam ring under various environment conditions (Carvalho and Santos, 2016).

For levofloxacin, according to the acceptability criteria mentioned in 3.2.6 (Ort et al., 2009), the $PECs_{inf}$ are acceptable ($0.5<PEC_{inf}/MEC_{inf\_avg}<2$) in both STPs, and the $PECs_{inf}$ calculated by the prescription volumes are more closed to $MECs_{inf}$. The predicted concentrations of clarithromycin on the basis of national shipping volume in STP B influent are unacceptably high (PEC_{inf}/MEC_{inf_avg}: 1.47-3.27), and those calculated by other sources in both STPs are belong to the acceptable level. For azithromycin, there was a big gap between PEC_{inf}/MEC_{inf_avg} based on national shipping data (0.95-3.18 in STP A, 0.87-2.92 in STP B) and those based on national prescription (0.10-0.33 in STP A, 0.09-0.30 in STP B)/regional prescription data (0.11-0.36 in STP A, 0.10-0.35 in STP B). The $PECs_{inf}$ calculated by the prescription volumes are unacceptably low, which might be caused by the statistical error for the NDB database. For levofloxacin, clarithromycin and azithromycin, the accuracy of estimation on the specific consumption in studied aera is the main factor influencing the accuracy of predicted results.

For sulfamethoxazole and trimethoprim, the $PECs_{inf}$ are lower than $MECs_{inf}$, and

the predicted concentrations of sulfamethoxazole are unacceptably low ($PEC_{inf}/MEC_{inf\_avg}<0.5$) in STP A, however, the $PECs_{inf}$ of sulfamethoxazole in STP B are acceptable; while it is acceptable for trimethoprim ($0.5<PEC_{inf}/MEC_{inf\_avg}<2$) in both STPs. It is worth noting that the $PECs_{inf}$ calculated by the regional prescription volumes are also more closed to $MECs_{inf}$. For these two compounds, the discrepancies found between $PECs_{inf}$ and $MECs_{inf}$ could be attributed to the underestimation of excretion rate, especially for sulfamethoxazole (15-20%) (Table 3-4).

From the above results, except azithromycin, the concentrations predicted from the prescription volumes are generally closer to the corresponding measured concentrations than those predicted from the shipping volumes. In addition, the $PECs_{inf}$ on the basis of regional prescription data for some compounds (e.g., ampicillin, piperacillin, cefazolin, clarithromycin, levofloxacin, sulfamethoxazole and trimethoprim in STP A, and ampicillin, piperacillin and clarithromycin in STP B) are more accurate. Azuma et al. (2015) also estimated the concentrations of seven target pharmaceuticals in sewage influents on the basis of two data sources (shipping volume and sales volume) and compared to MEC_{inf} in Japan, predicted concentrations on the basis of shipping data are generally tend to be higher than corresponding MEC. Compared the results of two STPs, the predicted scenarios in STP B are much closer to measured concentrations than those in STP A. There are several STPs served for the studied areas, and consumption bias of detected antibiotics might exist for the inhabitants served by the target STPs, the predicted results represent the average values in time and space, while the measured concentrations are typically related to a specific location and a certain point in time, which would be the main factors leading to the differences between the two STPs.

3.3.4 Predicted sewage influent concentrations applied for further estimation

The relationships between $PEC_{inf\_avg}/MEC_{inf\_avg}$ ratios on the basis of national shipping volumes and national/regional prescription volumes are shown in Figure 3-4.

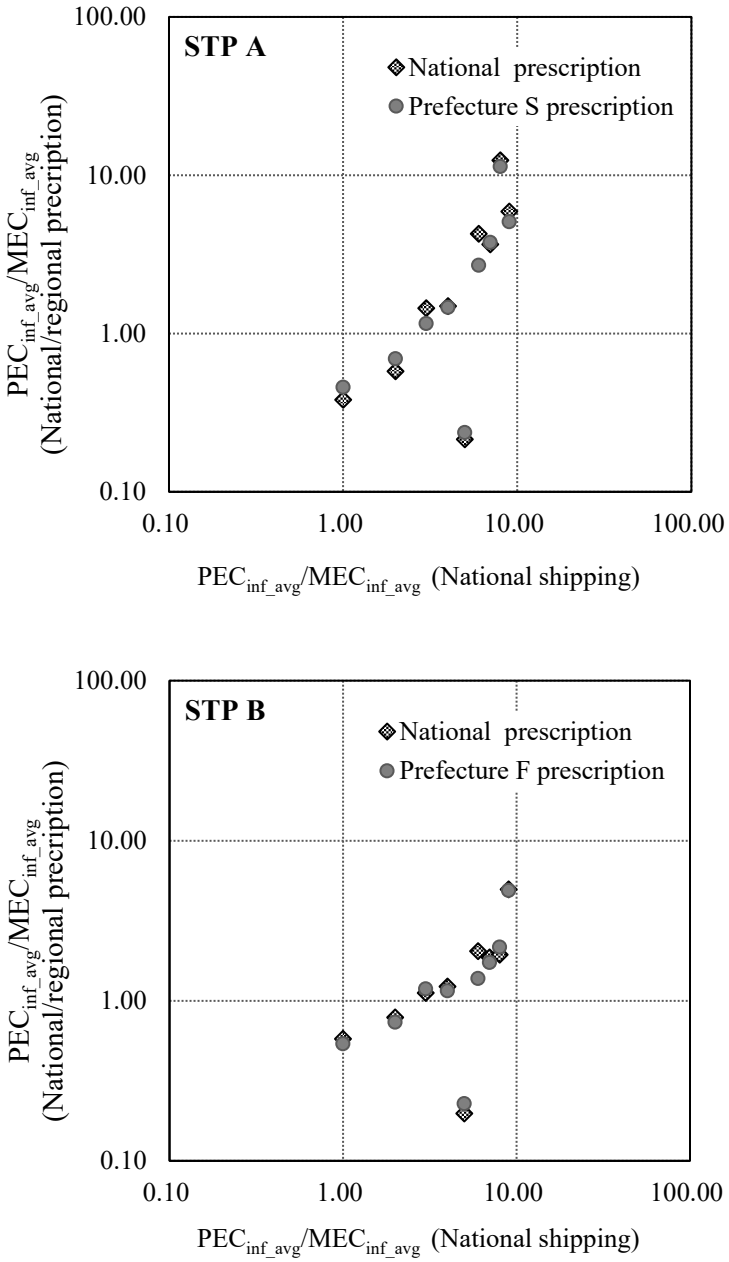


Figure 3-4 Relationship between the ratios of $PEC_{inf\_avg}/MEC_{inf\_avg}$ calculated by the national shipping volumes and national/regional prescription volumes in two STPs.

As discussed above, the accuracy of consumption volumes was an essential factor influencing the predicted concentrations. For most target compounds, the predicted concentrations are more closed to measured concentrations on the basis of regional prescription volumes. However, if there were some statistical errors exist in the databases, it would lead to unacceptably high or low consumption concentrations of

antibiotics, or data loss of some compounds. There were positive correlations between national shipping and national/regional prescription databases, and the correlation efficient (*r*) between the two are 0.84 and 0.82 in STP A, which are 0.72 and 0.73 in STP B, respectively. Therefore, it is possible to use the national shipping volumes to calculate the predicted concentrations when the statistical error existed on the regional prescription data of target compounds, but the predicted concentrations would be somewhat higher than those on the basis of national shipping data. As mentioned in 3.3.3, $PECs_{inf}$ of azithromycin calculated by the prescription volumes are unacceptably low, therefore, the $PECs_{inf}$ of azithromycin on the basis of national shipping volume replaces that of regional prescription volume for further estimation in STPs. For the other target antibiotics, $PECs_{inf}$ calculated by regional prescription data were applied for further estimation, which were summarized in Figure 3-5. For the compounds without analytical methods, the predicted data based on the regional prescription volume given in Table 3-6 would be used for further estimation. The present method for predicting sewage influent concentrations is simple and highly accurate, which provides a superiority for further estimation on the fate of target compounds in STPs.

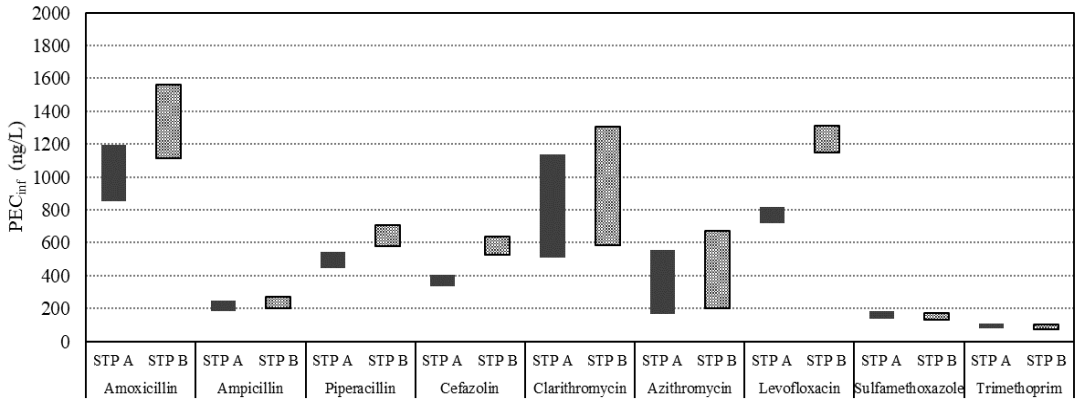


Figure 3-5 $PECs_{inf}$ of target antibiotics used for further estimation in two STPs.

3.3.5 Uncertainty analysis

Since the ratio of PEC_{inf}/MEC_{avg_inf} were used to evaluate the accuracy of prediction results. The annual report of conventional water quality parameters (BOD, COD and TN) in the target sewage influent showed that monthly average concentration varied between -46% to 24% with respect to the yearly average value, which means that

the $1/C_{inf}$ (concentration in sewage influent) varied from 0.8 to 1.9. Verlicchi et al. (2013) also reported that the trend of monthly consumption varied between -36% and +30 %, and antibiotics exhibit consumption peaks in some critical periods. Moreover, as antibiotics are micropollutants, the detected concentrations ranged from tens to thousands of ng/L in this study, the instrumental and human error might also have a high effect on the MEC. Therefore, the acceptable criteria range was set to 0.5-2 (shown in 3.2.6) based on these uncertainties.

For the prescription data, even we applied the prescription volume in related prefecture, the consumption bias still might exist since there are several STPs located in the prefecture. NDB covers around 98% of the prescription provided by health insurance, here we did not consider the prescription without health insurance, without electronic prescription or without prescription, which would lead to an underestimation of the predicted results. Unfortunately, we could not find any sources to estimate the percentage of these parts in Japan. While Safrany and Monnet (2012) estimated the percentage of antibiotics sold without prescription in 28 European countries and found a variation of 0 to 10% in different countries. Moreover, after prescription, some patients could not finish taking all medicine before recovery, just keep or discharge it as solid waste, the ignorance of this factor resulted in a certain extent of overestimation of prediction.

Additionally, since the excretion rate from human bodies varied by many factors, such as age, gender, health status and so on (Johnson and Williams, 2004; Verlicchi et al., 2014). Therefore, the maximum and the minimum excretion rate referred from literature were as extremes in this study, which was shown by the range of predicted concentrations.

3.4 Conclusions

In this Chapter, predicting equation based on consumption volumes of human-used antibiotics from two databases (shipping and prescription) had been applied to estimate the sewage influent concentrations of selected antibiotics in two STPs which located on different prefectures, and monitoring data (6 times between May, 2019 and March, 2020) were used to evaluate the accuracy of this predicting equation. The findings from

this study were as follows:

- ✓ Nine target compounds were measured in the sewage influent of two STPs. In STP A, the concentrations of target antibiotics in influents were ranged from 28 ng/L (ampicillin) to 1235 ng/L (levofloxacin), while in STP B, it was ranged from 35 ng/L (cefazolin) to 1471 ng/L (levofloxacin), and the seasonal fluctuation in STP B were relatively larger than that of STP A.
- ✓ The patterns of consumption of human-used antibiotics changed over the years. The shipping volumes of antibiotics are generally higher than prescription volumes. The amount of β-lactam antibiotics prescribed in Japan are the highest (around 50%), the prescription amounts of penicillins, which contain amoxicillin, ampicillin and piperacillin, represent for around 24% of total amounts of human-used antibiotics. The prescription volumes of macrolides, aminoglycosides and quinolones account for around 20%, 13% and 10%, representatively. In addition, the combination of sulfamethoxazole and trimethoprim represents for 5% of the total amount of antibiotics.
- ✓ The $PECs_{inf}$ of STP A were slightly lower than those of STP B based from the same data source, which is directly related to the per capita sewage production, 613 and 506 L/(capita·d), respectively. The predicted concentrations based on the national shipping data were slightly higher than those predicted by using national prescription data in the case of most antibiotics. In the two STPs, the highest predicted concentrations were estimated for levofloxacin and clarithromycin, with a range of concentrations between 512 and 1780 ng/L, followed by amoxicillin (563-1585 ng/L) and piperacillin (446-1080 ng/L).
- ✓ In two STPs, among the 9 detected antibiotics, 7 have a $PECs_{inf}$ calculated by national shipping volume greater than the corresponding $MECs_{inf}$, and 6 have a $PECs_{inf}$ calculated based on national/regional prescription volume greater than the corresponding $MECs_{inf}$. The $PECs_{inf}$ on the basis of prescription volumes are closer to $MECs_{inf}$ than those on the basis of shipping volume, but the predicted concentrations of azithromycin based on the prescription volumes were unacceptably low, which caused by human error of the NDB database.
- ✓ The accuracy of consumption volumes played an essential role during the

estimation. There were positive correlations between national shipping and national/regional prescription databases (correlation efficient r>0.70). Therefore, it is possible to use the national shipping volumes to calculate the predicted concentrations when the outlier existed based on the regional prescription data of target compounds, but the predicted concentrations would be somewhat higher than those on the basis of national shipping data. For the further estimation on the fate of target compounds in the STPs, the predicted concentrations of azithromycin were revised on the basis of national shipping volumes, and for the other compounds, the $PECs_{inf}$ calculated by the regional prescription data would be applied for further estimation.

3.5 Reference

Asai, T., Kojima, A., Harada, K., Ishihara, K., Takahashi, T. and Tamura, Y., 2005. Correlation between the usage volume of veterinary therapeutic antimicrobials and resistance in Escherichia coli isolated from the feces of food-producing animals in Japan. *Japanese journal of infectious diseases, 58*(6), pp.369.

Azuma, T., Nakada, N., Yamashita, N., Tanaka, H., 2015. Evaluation of concentrations of pharmaceuticals detected in sewage influents in Japan by using annual shipping and sales data. *Chemosphere*, *138*, pp.770-776.

Balbisi, E. A., 2002. Cefditoren, a new aminothiazolyl cephalosporin. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *22*(10), pp.1278-1293.

Baz-Lomba, J. A., Salvatore, S., Gracia-Lor, E., Bade, R., Castiglioni, S., Castrignanò, E., ... and McCall, A. K., 2016. Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. *BMC public health*, *16*(1), pp.1035.

Birošová, L., Mackuľak, T., Bodík, I., Ryba, J., Škubák, J., Grabic, R., 2014. Pilot study of seasonal occurrence and distribution of antibiotics and drug resistant bacteria in wastewater treatment plants in Slovakia. *Science of the Total Environment*, *490*, pp.440-444.

Brisson, A. M., Bryskier, A., Millerioux, L., Fourtillan, J. B., 1984. Pharmacokinetics of cefotiam administered intravenously and intramuscularly to healthy adults. *Antimicrobial Agents and Chemotherapy*, *26*(4), pp.513-518.

Bronzwaer, S.L., Cars, O., Buchholz, U., Mölstad, S., Goettsch, W., Veldhuijzen, I.K., Kool, J.L., Sprenger, M.J. and Degener, J.E., 2002. The relationship between antimicrobial use and antimicrobial resistance in Europe. *Emerging infectious diseases, 8*(3), pp.278.

Carballa, M., Omil, F., Lema, J. M., 2008. Comparison of predicted and measured concentrations of selected pharmaceuticals, fragrances and hormones in Spanish sewage. *Chemosphere*, *72*(8), pp.1118-1123.

Carvalho, I.T. and Santos, L., 2016. Antibiotics in the aquatic environments: a review of the European scenario. *Environment International, 94*, pp.736-75.

Dauphin, J. F., Graviere, C., Bouzard, D., Rohou, S., Chesne, C., Guillouzo, A., 1993. Comparative metabolism of tosufloxacin and BMY 43748 in hepatocytes from rat, dog, monkey and man. *Toxicology in vitro*, *7*(4), pp.499-503.

Ferrero, J. L., Bopp, B. A., Marsh, K. C., Quigley, S. C., Johnson, M. J., Anderson, D. J., Lamm, J. E., Tolman, K. G., Sanders, S. W., Cavanaugh, J. H., 1990. Metabolism and disposition of clarithromycin in man. *Drug Metabolism and Disposition*, *18*(4), pp.441-446.

Foulds, G., Shepard, R. M., Johnson, R. B., 1990. The pharmacokinetics of azithromycin in human serum and tissues. *Journal of Antimicrobial Chemotherapy*, *25*(suppl_A), pp.73-82.

Fujimori K., 2016. Current status and issues of the national database (in Japanase). *Journal of Health and Social Behaviors*. *26*, pp.15-24.

Gavazzi, G., Krause, K. H., 2002. Ageing and infection. *The Lancet infectious diseases*, *2*(11), pp.659-666.

Ghosh, G., Hanamoto, S., Yamashita, N., Huang, X., Tanaka, H., 2016. Antibiotics removal in biological sewage treatment plants. *Pollution*, *2*(2), pp.131-139.

Harrison, M. P., Moss, S. R., Featherstone, A., Fowkes, A. G., Sanders, A. M., Case, D. E., 1989. The disposition and metabolism of meropenem in laboratory animals and man. *Journal of Antimicrobial Chemotherapy*, *24*(suppl_A), pp.265-277.

Hayashi, Y., Roberts, J. A., Paterson, D. L., Lipman, J., 2010. Pharmacokinetic evaluation of piperacillin-tazobactam. *Expert Opinion on Drug Metabolism & Toxicology*, *6*(8), pp.1017-1031.

Heberer, T., 2002. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicology letters, 131*(1-2), pp.5-17.

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

Karthikeyan, K. G. and Meyer, M. T., 2006. Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *Science of the Total Environment, 361*(1-3), pp.196-207.

Ko, H. O. W. A. R. D., Cathcart, K. S., Griffith, D. L., Peters, G. R., Adams, W. J., 1989. Pharmacokinetics of intravenously administered cefmetazole and cefoxitin and effects of probenecid on cefmetazole elimination. *Antimicrobial Agents and Chemotherapy*, *33*(3), pp.356-361.

Kümmerer, K., Henninger, A., 2003. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clinical Microbiology and Infection*, *9*(12), pp.1203-1214.

Le-Minh, N., Khan, S.J., Drewes, J.E. and Stuetz, R.M., 2010. Fate of antibiotics during municipal water recycling treatment processes. *Water research*, *44*(15), pp.4295-4323.

Narumiya, M., Nakada, N., Yamashita, N., Tanaka, H., 2013. Phase distribution and removal of pharmaceuticals and personal care products during anaerobic sludge digestion. *Journal of Hazardous Materials*, *260*, pp.305-312.

Neu, H. C., 1974. Antimicrobial activity and human pharmacology of amoxicillin. *Journal of Infectious Diseases*, *129*(Supplement_2), pp.S123-S131.

Opriş, O., Soran, M. L., Coman, V., Copaciu, F., Ristoiu, D., 2013. Determination of some frequently used antibiotics in waste waters using solid phase extraction followed by high performance liquid chromatography with diode array and mass spectrometry detection. *Central European Journal of Chemistry*, *11*(8), pp.1343-1351.

Ort, C., Hollender, J., Schaerer, M., Siegrist, H., 2009. Model-based evaluation of reduction strategies for micropollutants from wastewater treatment plants in complex river networks. *Environmental Science & Technology*, *43*(9), pp.3214-3220.

Patel, I. H., Chen, S., Parsonnet, M., Hackman, M. R., Brooks, M. A., Konikoff, J., Kaplan, S. A., 1981. Pharmacokinetics of ceftriaxone in humans. *Antimicrobial Agents and Chemotherapy*, *20*(5), pp.634-641.

Pérez, S., Eichhorn, P., Aga, D. S., 2005. Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. *Environmental Toxicology and Chemistry: An International Journal*, *24*(6), pp.1361-1367.

Rattie, E. S., Ravin, L. J., 1975. Pharmacokinetic interpretation of blood levels and urinary excretion data for cefazolin and cephalothin after intravenous and intramuscular administration in humans. *Antimicrobial Agents and Chemotherapy*, *7*(5), pp.606-613.

Riva, F., Zuccato, E., Castiglioni, S., 2015. Prioritization and analysis of pharmaceuticals for human use contaminating the aquatic ecosystem in Italy. *Journal of Pharmaceutical and Biomedical Analysis*, *106*, pp.71-78.

Safrany, N., Monnet, D. L., 2012. Antibiotics obtained without a prescription in Europe. *The Lancet Infectious Diseases*, *12*(3), pp.182-183.

Sato, K., Watanabe, H., Ikeda, T., Miida, H., Ohtaki, K. and Watanabe, K., 2018. Estimation of total prescription weights of active pharmaceutical ingredients in human medicines based on a public database for environmental risk assessment in Japan. *Regulatory Toxicology and Pharmacology*, *99*, pp.98-104.

Schröder, H., Campbell, D. E., 1972. Absorption, metabolism, and excretion of salicylazosulfapyridine in man. *Clinical Pharmacology & Therapeutics*, *13*(4), pp.539-551.

Spyker, D. A., Thomas, B. L., Sande, M. A., Bolton, W. K., 1978. Pharmacokinetics of cefaclor and cephalexin: dosage nomograms for impaired renal function. *Antimicrobial Agents and Chemotherapy*, *14*(2), pp.172-177.

Statistic Bureau, Japan. Statistical Handbook of Japan, 2014-2016. https://www.stat.go.jp/data/s-sugata/index.html

Totsuka, K., Shimizu, K., Konishi, M., Yamamoto, S., 1992. Metabolism of S-1108, a new oral cephem antibiotic, and metabolic profiles of its metabolites in humans. *Antimicrobial Agents and Chemotherapy*, *36*(4), pp.757-761.

Van De Sande-Bruinsma, N., Grundmann, H., Verloo, D., Tiemersma, E., Monen, J., Goossens, H., Ferech, M. and European Antimicrobial Resistance Surveillance System, 2008. Antimicrobial drug use and resistance in Europe. *Emerging infectious diseases, 14*(11), pp.1722.

Verlicchi, P., Al Aukidy, M., Jelic, A., Petrović, M., Barceló, D., 2014. Comparison of measured and predicted concentrations of selected pharmaceuticals in wastewater and surface water: a case study of a catchment area in the Po Valley (Italy). *Science of the Total Environment*, *470*, pp.844-854.

Verlicchi, P., Galletti, A., Petrovic, M., Barceló, D., Al Aukidy, M., Zambello, E., 2013. Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed—analysis of their respective contributions. *Science of the Total Environment*, *454*, pp.411-425.

Watkinson, A.J., Murby, E.J. and Costanzo, S.D., 2007. Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. *Water research, 41*(18), pp.4164-4176.

Xu, W., Zhang, G., Li, X., Zou, S., Li, P., Hu, Z. and Li, J., 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. *Water research*, *41*(19), pp.4526-4534.

Yasojima, M., Nakada, N., Komori, K., Suzuki, Y., Tanaka, H., 2006. Occurrence of levofloxacin, clarithromycin and azithromycin in wastewater treatment plant in Japan. *Water Science and Technology*, *53*(11), pp.227-233.

Yilmaz, M., Paterson, D. L., 2010. Ceftizoxime, cefdinir, cefditoren, cefpodoxime, ceftibuten, cefsulodin, and cefpiramide. En: Grayson ML, Crowe SM, McCarthy JS, Mills J, Mouton JW, Norrby SR, et al., comp. Kucers' The use of antibiotics, 6th ed. London: Hodder-Arnold, pp.390-397.

Chapter IV

Comparison on the removal performances of antibiotics in anaerobic/anoxic/oxic (AAO) sludge, conventional activate sludge (CAS) and membrane bioreactor (MBR) sludge

4.1 Introduction

Biological sewage treatment plants play a crucial role in the reduction of the risk by discharging antibiotics in the receiving environmental bodies. Conventional activated sludge (CAS) process has been widely used for municipal sewage treatment. However, it was considered insufficient for the removal of antibiotics. It has been reported that anaerobic/anoxic/oxide (A/A/O) process and membrane bioreactor (MBR) showed higher removal performances compared to CAS process (Clara et al., 2005; Xue et al., 2010). Biodegradation and adsorption are reported as the main removal pathways in STPs; however, it is still not clear that why MBR process and A/A/O process are more effective in the biodegradation and adsorption than CAS. The sorption distribution coefficients (K_d) of antibiotics in the sludge and biodegradation kinetic constants of target antibiotics in different activated sludge systems are essential parameters for better understanding of antibiotics removal mechanisms and further estimation on the removal of antibiotics during the tertiary treatment steps.

K_d is the ratio of the equilibrium concentration of chemical on the solids to the corresponding equilibrium aqueous concentration. Due to the limitation of detecting methods or experimental condition, the experimental data of some compounds are not available or accurate (Stevens-Garmon et al., 2011). Previous studies have reported some empirical models for the sorption of micropollutants to sludge, however, the empirical constants varied by different studies (Hyland et al., 2012; Lakshminarasimman et al., 2018; Stevens-Garmon et al., 2011). Therefore, it is

important to apply proper empirical constants to estimate the K_d values by experimental data.

In the most previous studies, the biodegradation experiments of micropollutants were estimated using first-order kinetics (Lakshminarasimman et al., 2018; Mazioti et al., 2015; Plósz et al., 2010). However, it has been clarified that ammonia-oxidizing bacteria (AOB) can catalyze the oxidation of antibiotics via Ammonia monooxygenase (AMO) enzyme (cometabolism) (Kassotaki et al., 2016; Kumwimba and Meng, 2019; Park et al., 2017), and the cometabolism degradation rate of antibiotics was expressed by zero-order kinetics, which would result in different kinetics in AOB enriched systems (e.g., MBR). Therefore, it is essential to characterize the contribution of AOB cometabolism on the removal of target antibiotics and separately estimate the kinetic rate constants. In addition, the microbial diversity and composition varied in different activated sludge systems, which would also influence the removal of antibiotics (Petrie et al., 2014).

In this Chapter, the K_d values of target antibiotics were determined, which were applied to assess the empirical constants of K_d estimation models. Meanwhile, the biotransformation kinetics of target compounds in three different activated sludge systems were characterized, and the contribution of AOB cometabolism on their removal were also evaluated. There were two different biodegradation kinetics applied to estimate the biotransformation kinetic constants of target antibiotics: 1) first-order kinetics; 2) zero-order kinetics for AOB cometabolism and first-order kinetics for the rest degradation, and the effectiveness of these two kinetics were assessed.

4.2 Materials and Methods

4.2.1 Materials

There are nine target antibiotics for batch experiments (amoxicillin, ampicillin, piperacillin, cefazolin, azithromycin, clarithromycin, levofloxacin, sulfamethoxazole and trimethoprim). The information on the chemicals and reagents are according to 3.2.1. Activated sludges for the batch experiments were taken from the biological tank of STP A, STP B and the demonstrated-MBR tank of the STP B (Figure 4-1). The

activated sludges were transported to the laboratory and processed within two hours. The SRT of STP A is 14 (±2) days, with a MLSS concentrations of 2000-3000 mg/L, and the SRT and MLSS concentrations in STP B are 14 (±2) days and 1100-1400 mg/L, representatively. For the demonstrated-MBR tank, SRT is 43 (±2) days and MLSS concentrations are 8000-12000 mg/L.

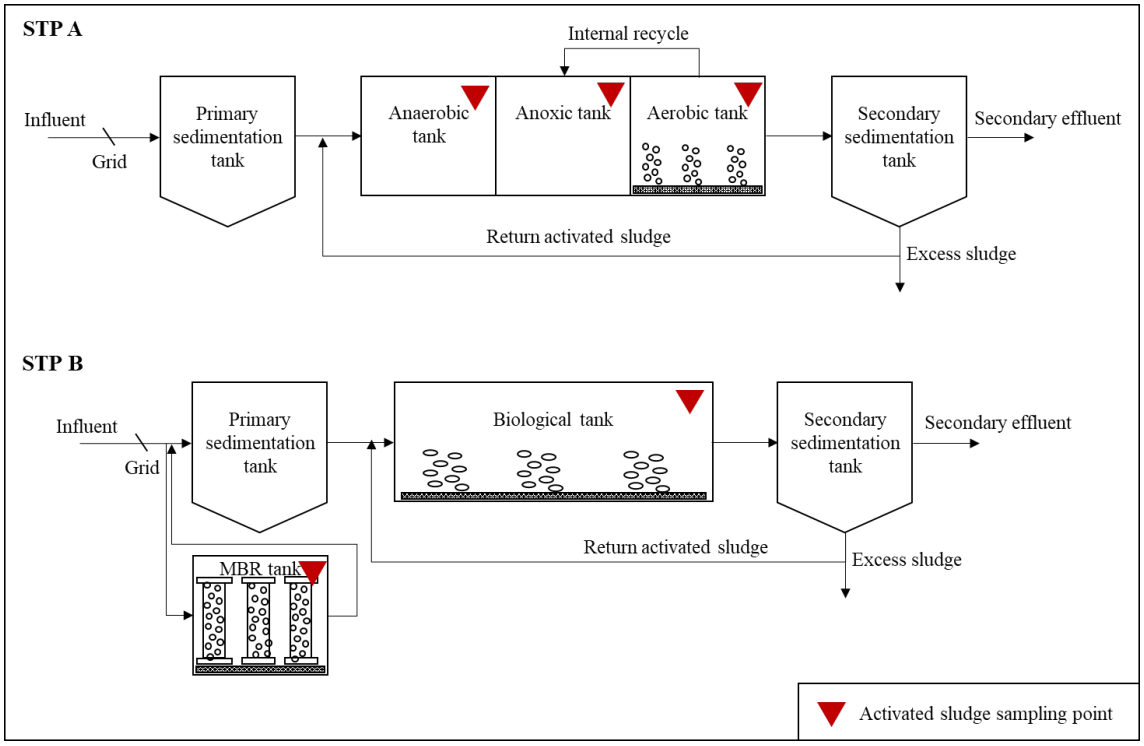


Figure 4-1 Biological treatment flows of two target STPs and activated sludge sampling point for batch experiments

4.2.2 Batch experiments

Batch experiments were carried out to evaluate the removal pathways of 9 target antibiotics and the contribution of AOB cometabolism. After taking the activated sludge samples to the laboratory, the sludge was washed for three times: after centrifugation, the supernatant was discharged and re-suspended by deionized water to the same volume, this procedure was replicated for three times to remove the compounds in the liquid phase of the activated sludge. 3 L of activated sludge were separated to three brown glass bottles with magnetic stirrers and aeration stones. One was used as biotransformation reactor (RUN 1). In the case of RUN 2, 5g/L of sodium azide (NaN_3)

was added to inactive the bioactivity of microorganisms (Xu et al., 2008). The supplementary experiments confirmed that 0.5 % NaN_3 could completely inhibit the bioactivity of activated sludge. Allylthiourea (ATU) is a well-known inhibitor of AMO (a membrane-bound enzyme used by AOB to catalyze the oxidation of NH_3 to hydroxylamine) and has been used in many studies to inhibit the activity of AOB (Kassotaki et al., 2016; Roh et al., 2009). To evaluate to contribution of AOB cometabolism on the removal of each target compound, 30 mg/L of ATU was added to inhibit the activity of AOB (RUN 3). The control group was also set with Milli-Q water as RUN 4. For the activated sludge taken from STP A, RUN 5-6 were set to evaluate the removal of target compounds under anoxic and anaerobic conditions, the nitrogen gas was blowing in the activated sludge for 30 min before the experiment to remove the oxygen. Due to the decreasing of pH during nitrification process, $NaHCO_3$ were added to adjust the alkalinity according to the NH_4-N: $NaHCO_3$ ratio of 1:18. A nutrient solution was spiked to each RUN with the components of 50 mg/L Na_2HPO_4, 70 mg/L $CaCl_2$, 400 mg/L $MgSO_4$ and 0.4 mL/L of trace element solutions. The specific components of trace element solution were prepared according to Smolders et al. (1994). The fed ammonium in RUN 1-4 was 20 mg NH_4^+-N/L, which was 5 mg NH_4^+-N/L in RUN 5-6. The glucose solution was spiked to each RUN, with an initial COD concentration of 100 mg COD/L. In RUN 5, NO_3^--N was spiked with an initial concentration of 20 mg NO_3^--N/L for denitrification. All RUNs have an initial individual antibiotic mass of 100 μg. With the exception of RUN 5-6, the dissolved oxygen (DO) concentrations were maintained higher than 4 mg/L. For all reactors, 20 mL of samples were collected after 0, 1, 2, 4, 8, 12, 24, and 48 h, 10 ml of sample was filtered through the glass fiber filter (Whatman GF/B, 1 μm) for the analysis of MLSS, MLVSS, NH_4^+-N, NO_2^--N, NO_3^--N, and dissolved organic carbon (DOC) concentration, and the other 10 ml of sample was used to measure the target antibiotics concentration in both liquid and solid phase.

4.2.3 Analytical methods

NH_4^+-N, NO_2^--N and NO_3^--N/L concentrations were measured by ion chromatography (Dionex Aquion, Thermo Fisher Scientific). DOC concentration was

measured with a TOC-300V analyzer (Mitsubishi Chemical Analytech Kanagawa, Japan). MLSS and MLVSS concentrations were measured by standard method according to APHA (2007). Target antibiotics concentration was analyzed according to 3.2.3. DO concentrations were determined by a DO meter (DO-24P, DKK·TOA Corporation, Japan).

4.2.4 Calculations equations

4.2.4.1 Sorption distribution coefficient (K_d)

Sorption distribution coefficient (K_d, $L \cdot kg^{-1}$) of each target compound was calculated by following equation:

$$K_d = \frac{C_S/(\mathrm{MLSS} \times 10^{-3})}{C_{aq}} \qquad \text{(Equation 4-1)}$$

where, MLSS is the concentration of mixed liquor suspended solids ($g \cdot L^{-1}$), C_S is the target compounds concentration in the solid phase ($ng \cdot L^{-1}$), C_{aq} is the target compounds concentration in the liquid phase ($ng \cdot L^{-1}$).

Schwarzenbach et al. (2003) proposed that the K_d values of micropollutants (including antibiotics) can be expressed by following equation:

$$K_d = f_{oc} K_{oc} \qquad \text{(Equation 4-2)}$$

where f_{oc} is the fraction of organic carbon present on the sludge (kg_{oc}/kg_{SS}), based on the composition of primary sedimentation sludge and activated sludge (McCarty, 1974; Namkung and Rittmann, 1987), f_{oc_prim}=0.597, f_{oc_act} and f_{oc_secd} =0.531; and K_{oc} is the organic-carbon distribution coefficient (L/kg_{oc}). The linear free energy relationships (LFERs) have been applied in some previous studies to estimate K_{oc} (Hyland et al., 2012; Lakshminarasimman et al., 2018; Stevens-Garmon et al., 2011) (Equation 4-3):

$$\log K_{oc} = a \log K_{ow} + b \qquad \text{(Equation 4-3)}$$

where K_{ow} is the octanol-water partitioning coefficient, a and b are constants from empirical data, which are 0.79 and 0.47 in Hyland et al. (2012), 0.6 and 0.69 in Stevens-Garmon et al. (2011). The experimental K_d values of antibiotics were applied to evaluate the predictive models for partitioning based on log K_{ow}.

4.2.4.2 First-order kinetic model

Numerous previous studies applied the pseudo first-order kinetic model to estimate the biodegradation of antibiotics (RUN 1), which is the simplified version of the Monod-model. When the substrate concentration is significantly lower than the half-saturation coefficient, the biomass transformation capacity increases linearly with the substrate concentration (Equation 4-4),

$$\frac{dC}{dt} = -k_{bio} \cdot X_S \cdot C \leftrightarrow C_t = C_0 \cdot e^{-k_{bio} \cdot X_S \cdot t} \qquad \text{(Equation 4-4)}$$

Where, k_{bio} is the first-order biodegradation rate constant normalized to mixed liquor volatile suspended solid, which better represents the biomass concentration ($L \cdot gVSS^{-1} \cdot d^{-1}$). C_t and C_0 are the target compounds concentrations in batch experiment at time t and t=0, respectively, (ng/L). X_S is the VSS (g/L). Using this equation, the half-lives (h) can be calculated as $(\ln 2)/(k_{bio} \cdot X_S)$.

4.2.4.3 Separately characterization on AOB cometabolic kinetics

Except for AOB cometabolism, the rest part of biodegradation (RUN 3) was still estimated by the first-order kinetic model (Equation 4-4), k_{bio}' ($L \cdot gVSS^{-1} \cdot d^{-1}$) value was estimated. (RUN1-RUN3) represents the antibiotics degradation by AOB cometabolism, and the AOB cometabolism on target antibiotics was estimated by zero-order kinetic model (Equation 4-5):

$$\frac{dC}{dt} = -k_{AOB} \cdot X_S \leftrightarrow C_t = C_0 - k_{AOB} \cdot X_S \cdot t \qquad \text{(Equation 4-5)}$$

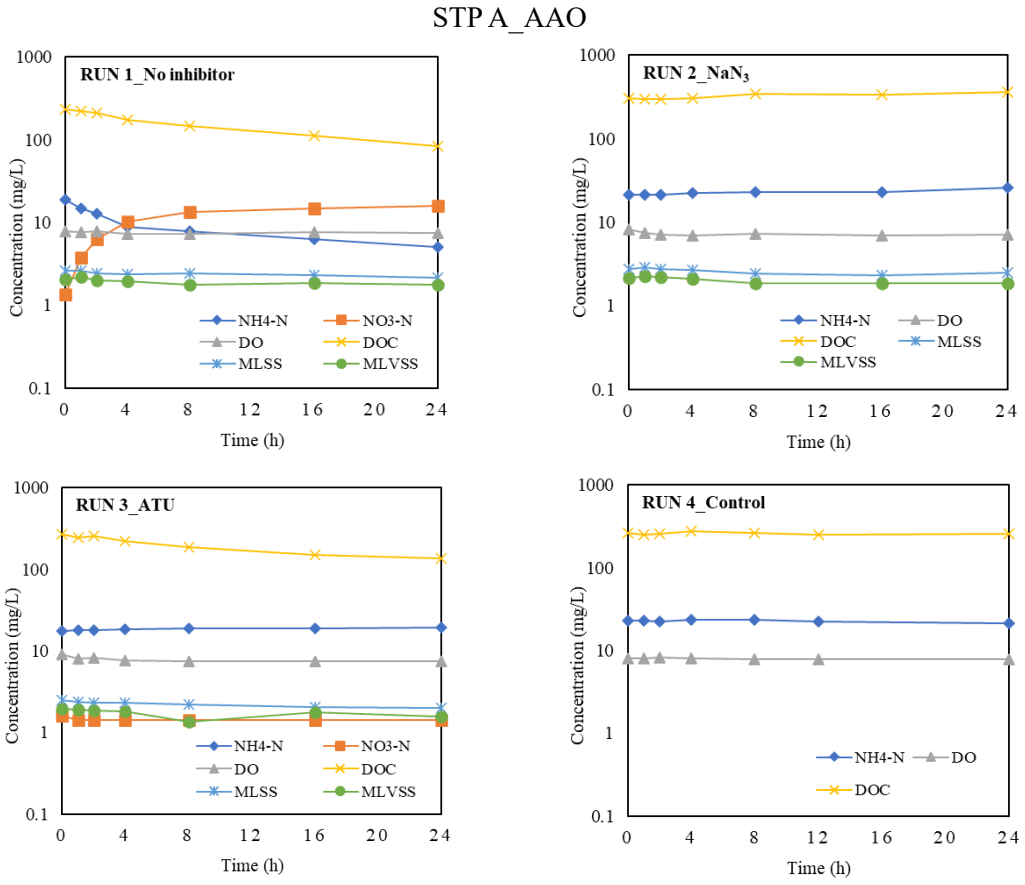
Where, k_{AOB} is the zero-order AOB cometabolism rate constant normalized to VSS ($ng \cdot g\ VSS^{-1} \cdot d^{-1}$). The k_{AOB} value was predicted by minimizing $\sum(C_{RUN1\_t} - C_{pred\_t})$, C_{pred_t} is total concentration at time t calculated by predicted k_{AOB} ($\mu g \cdot g\ VSS^{-1} \cdot d^{-1}$) value and k_{bio}' ($L \cdot gVSS^{-1} \cdot d^{-1}$) value. The activity of AOB (q_{AOB}) in the activated sludge was characterized by the specific NH_4^+-N utilization rate ($mg\ N \cdot gVSS^{-1} \cdot d^{-1}$). The contribution of AOB was calculated by the Equation 4-6:

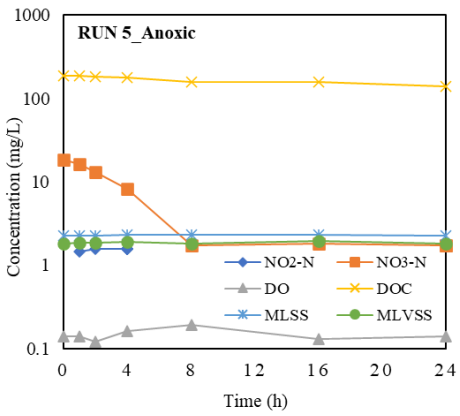
$$Contribution_{AOB} = \frac{\text{Degradation volume by AOB (ng/L)}}{\text{Total degradation volume (ng/L)}} \times 100\% \qquad \text{(Equation 4-6)}$$

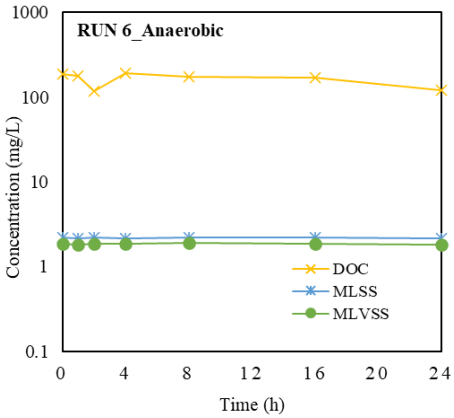
4.3 Results and Discussions

4.3.1 Analysis of conventional parameters

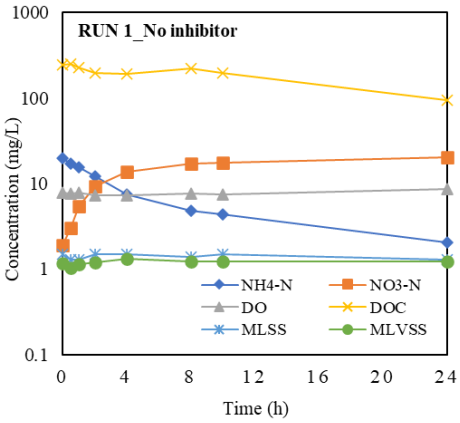
The conventional parameter results in biotransformation batch experiments are shown in Figure 4-2. For the groups of 0.5% NaN_3 addition, there was no utilization of NH_4-N, and the DOC concentrations were not decreased by time. In the groups of ATU addition, NH_4-H concentrations were not decreased to the end of batch experiments, while the utilization rates of DOC were comparable to those of no inhibitor addition groups. In the RUN 1-4, DO concentrations were all above 4 mg/L during the experiments. It reveals that the operating conditions were controlled properly during the batch experiments. According the utilization rate of NH_4-N in RUN 1, the activity of AOB in activated sludge taking from STP A_Aerobic, STP B_CAS and STP B_MBR were 16.22, 36.16 and 26.29 mg N/(gVSS·d), respectively.

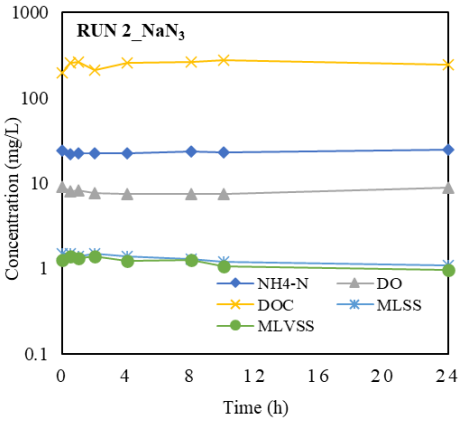


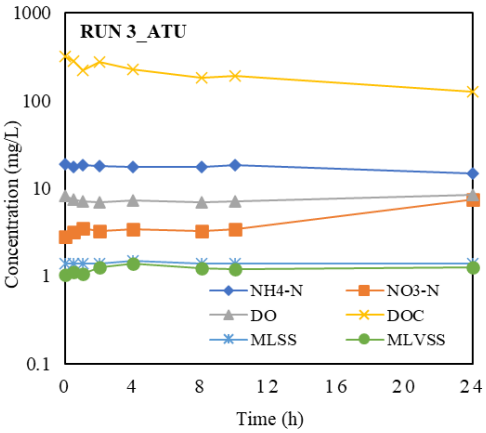


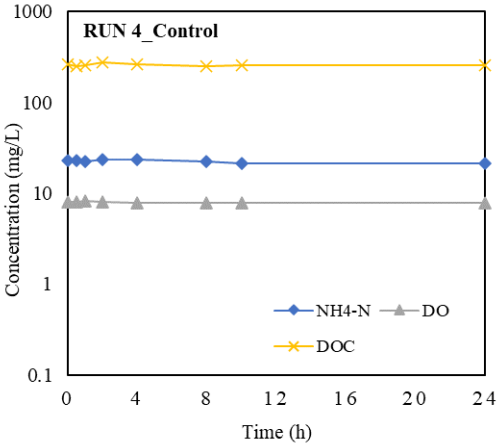


STP B_CAS









STP Demonstrated MBR

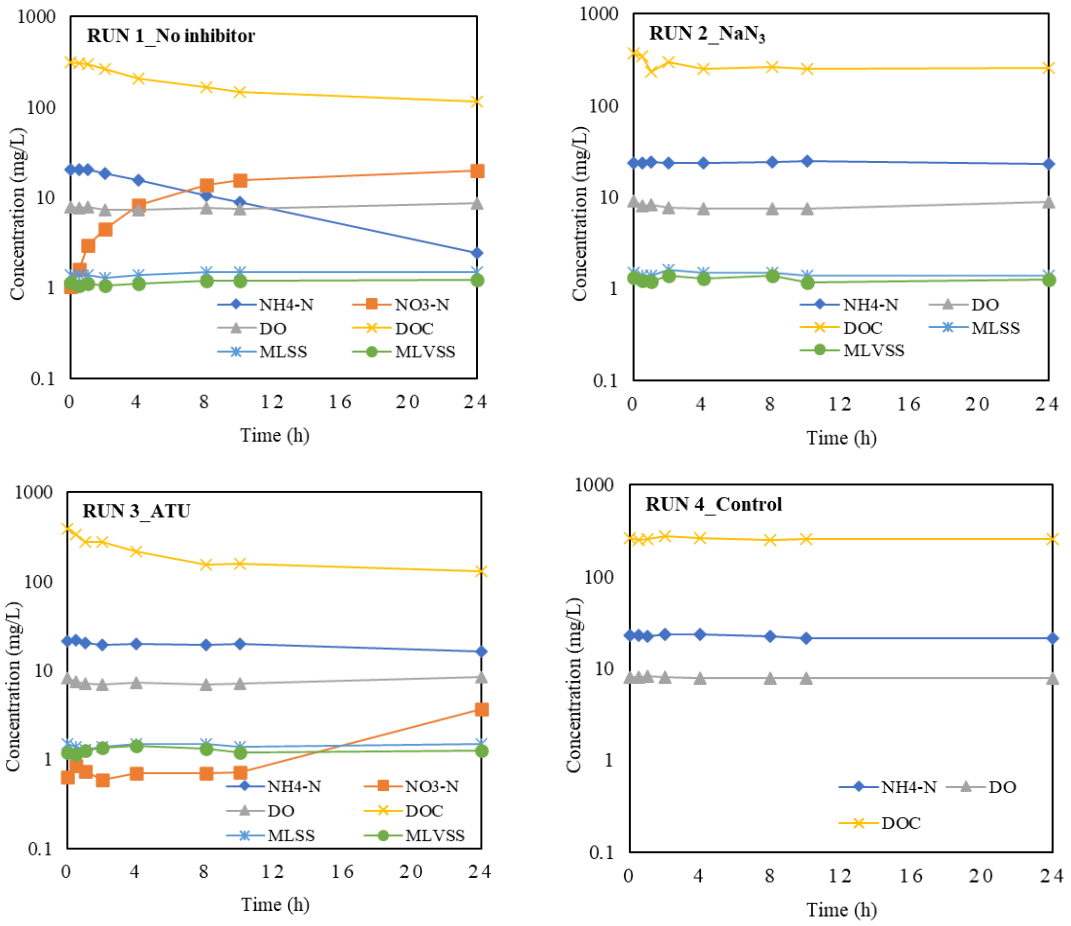


Figure 4-2 Conventional parameter results in biotransformation batch experiments

4.3.2 Sorption distribution coefficients

The sorption distribution coefficients (K_d) of 9 selected antibiotics were determined, the results and some related data from literature are shown in Table 4-1. These compounds were classified based on the average log K_d values to lowly sorptive ($log\ K_d$ <2), moderately sorptive (2 < $log\ K_d$ < 3) and highly sorptive ($log\ K_d$ > 3) and discussed separately below.

4.3.2.1. Lowly sorptive (log K_d < 2)

All β-lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), sulfamethoxazole and trimethoprim were classified as lowly sorptive. The K_d values of β-lactams (amoxicillin, ampicillin, piperacillin and cefazolin) ranged from 2.4 to 28.4 L/kg (log K_d =0.39-1.45), the average log K_d value (n=5) of these four compounds for all redox conditions and sludges from all STPs were 1.00 ± 0.12, 0.98 ± 0.13, 0.83 ±

0.06, and 0.66 ± 0.12, respectively. There was little report on the K_d values of β-lactam antibiotics in previous studies, Blair et al. (2015) reported similar sorption distribution coefficient of ampicillin (30 ± 17 L/kg) in conventional activated sludge. The K_d values of sulfamethoxazole ranged from 12 to 131 L/kg in all conditions with an average log K_d value (n=5) of 1.67 ± 0.24, which were similar to the value of 16.7 L/kg reported by Kazama (2017) and 40 ± 13 L/kg reported by Abegglen et al. (2009). Some studies reported slightly lower sorption distribution coefficients (188-241 L/kg and 256 ± 169 L/kg) (Göbel et al., 2005; Lakshminarasimman et al., 2018), and Blair et al. (2015) reported much lower sorption distribution coefficients (10 ± 9 L/kg). The K_d values of trimethoprim ranged from 48 to 134 L/kg, and the average log K_d value (n=5) in all conditions were 1.91 ± 0.12, which were similar to the value 208 ± 49 L/kg reported by Göbel et al. (2005), and the value 71.7 L/kg reported by Kazama (2017). Blair et al. (2015) reported much lower sorption distribution coefficients (14 ± 6 L/kg).

4.3.2.2. Moderately sorptive (2 < log K_d < 3)

Clarithromycin and azithromycin were classified as moderately sorptive. The K_d values for clarithromycin ranged from 236 to 1315 L/kg (log K_d =2.73 ± 0.13, n=5), which were similar to the value of 730 ± 50 L/kg reported by Abegglen et al. (2009), and the value of 794 L/kg reported by Narumiya (2011). Other studies reported slightly lower sorption distribution coefficients (< 262 L/kg) (Blair et al., 2015; Göbel et al., 2005;) Kazama, 2017). The K_d values for azithromycin ranged from 284 to 858 L/kg with an average log K_d value (n=5) in all condition of 2.65 ± 0.11, which were similar to the value 376 ± 86 L/kg reported by Göbel et al. (2005), and the value 794 L/kg reported by Narumiya (2011). Some studies reported much lower sorption distribution coefficients (< 130) L/kg (Blair et al., 2015; Kazama, 2017).

4.3.2.3. Highly sorptive (log K_d > 3)

Levofloxacin was the only compound classified as highly sorptive in this study, the K_d values ranged from 272 to 6484 L/kg (log K_d =3.00 ± 0.34, n=5), which were similar to the value 5330 L/kg reported by Kazama (2017), and the value 1259 L/kg reported by Narumiya (2011).

Table 4-1 Sorption distribution coefficients in activated sludge collected from STP A_AAO, STP B_CAS and STP B_MBR in this study (average concentration of K_d ± SD), and related data from literatures are provided for comparison.

	STP A_AAO						STP B_CAS		STP B_MBR		Literature
	Aerobic		Anoxic		Anaerobic						
	K_d (L/kg)	logK_d	K_d (L/kg)	logK_d	K_d (L/kg)	logK_d	K_d (L/kg)	logK_d	K_d (L/kg)	logK_d	K_d (L/kg)
Amoxicillin	7.7 ± 1.2	0.88	16.6 ± 8.4	1.16	15.1 ± 7.2	1.12	7.9 ± 1.8	0.89	9.6 ± 1.8	0.97	NA
Ampicillin	6.8 ± 0.4	0.83	15.1 ± 1.8	1.14	13.5 ± 4.0	1.13	7.7 ± 1.5	0.88	8.0 ± 0.9	0.90	30 ± 17 (CAS)[a]
Piperacillin	5.7 ± 0.4	0.76	8.5 ± 0.9	0.92	7.1 ± 1.1	0.85	6.8 ± 1.4	0.82	6.2 ± 1.6	0.78	NA
Cefazolin	2.6 ± 0.1	0.42	6.1 ± 0.6	0.74	5.5 ± 1.2	0.74	5.7 ± 1.7	0.73	5.0 ± 0.9	0.69	NA
Clarithromycin	459 ± 99	2.69	1010 ± 915	2.90	948 ± 593	2.91	547 ± 167	2.72	585 ± 187	2.74	130 ± 320 (CAS)[a], 262 ± 93 (CAS)[b], 62.2 (MBR)[c], 730 ± 50 (MBR)[d], 794 (AAO)[e]
Azithromycin	390 ± 86	2.58	800 ± 550	2.83	928 ± 638	2.89	416 ± 90	2.61	465 ± 111	2.65	130 ± 280 (CAS)[a], 376 ± 86 (CAS)[b], 59.1 (MBR)[c], 794 (AAO)[e]
Levofloxacin	532 ± 287	2.67	2174 ± 994	3.30	2772 ± 1684	3.37	613 ± 181	2.76	584 ± 217	2.74	5330.0 (MBR)[c], 1259 (AAO)[e]
Sulfamethoxazole	54 ± 10	1.72	44 ± 22	1.60	24 ± 10	1.35	91 ± 57	1.89	76 ± 28	1.85	10 ± 9 (CAS)[a], 256 ± 169 (CAS)[b], 16.7 (MBR)[c], 40 ± 13 (MBR)[d]
Trimethoprim	63 ± 10	1.79	81 ± 18	1.90	90 ± 24	1.94	91 ± 23	1.95	95 ± 22	1.97	14 ± 6 (CAS)[a], 208 ± 49 (CAS)[b], 71.7 (MBR)[c]

NA: not available

References: a) Blair et al., 2015; b) Göbel et al., 2005; c) Kazama, 2017; d) Abegglen et al., 2009; e) Narumiya, 2011.

4.3.2.4. Impact of redox condition and operating system

The regression plots of log K_d values in different redox conditions of STP A_AAO are given in Figure 4-3. Variability of sulfamethoxazole in log K_d values were more than those of other compounds. There were high correlations of log K_d values between different redox conditions ($R^2 > 0.9$), and the highest correlation was between anoxic and anaerobic activated sludge ($R^2 = 0.993$), which was coincident with the results reported by Lakshminarasimman et al. (2018). All three plots also showed high correlation ($R^2 > 0.98$) between different treatment systems, and the highest correlation was between STPB_CAS and STPB_MBR activated sludge (Figure 4-4). Both redox condition and treatment system could affect the microbial composition of activated sludge (Vuono et al., 2015; Xu et al., 2010), which would influence the sludge property and sorption capacity. The differences between the regression plots and y=x indicates that the redox conditions affect the sorption capacity of sludge more than that of treatment system.

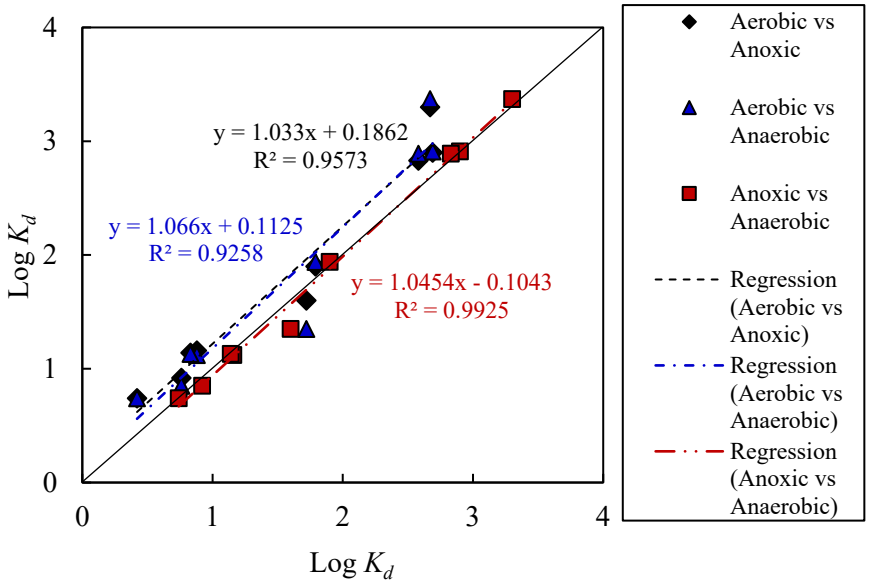


Figure 4-3 Relationship of log K_d values between different redox conditions: Aerobic and Anoxic (Anoxic log K_d =1.033×Aerobic log K_d + 0.1862, R^2=0.954, n=9), Aerobic and Anaerobic (Anaerobic log K_d =1.066×Aerobic log K_d + 0.1125, R^2=0.926, n=9), Anoxic and Anaerobic (Anaerobic log K_d =1.0454×Anoxic log K_d - 0.1043, R^2=0.993, n=9) in STPA_AAO.

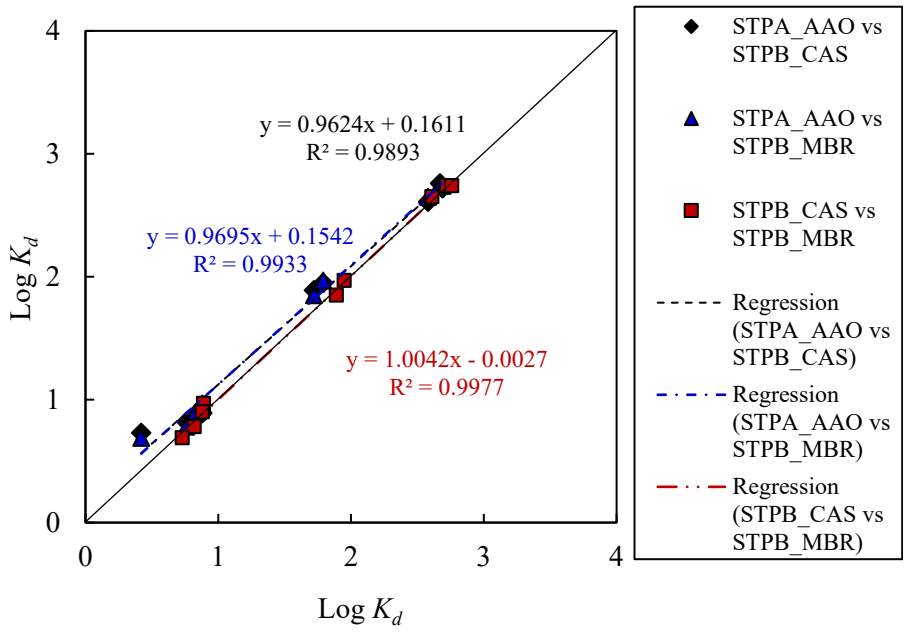


Figure 4-4 Relationship of log K_d values between different operating systems (aerobic activated sludge): STPA_AAO and STPB_CAS (STPA_AAO log K_d =0.9624×STPB_CAS log K_d + 0.1611, R^2=0.989, n=9), STPA_AAO and STPB_MBR (STPA_AAO log K_d =0.9695×STPB_MBR log K_d + 0.1542, R^2=0.993, n=9), STPB_CAS and STPB_MBR (STPB_CAS log K_d =1.0042×STPB_MBR log K_d - 0.0027, R^2=0.998, n=9)

4.3.2.5. Evaluation on empirical predictive models of sorption distribution coefficient

According to Equation 4-2 and 4-3 in 4.2.4, the empirical constants (*a* and *b*) of the predictive model were calculated based on the measured sorption distribution coefficients (log K_d) and octanol-water partitioning coefficient (log K_{ow}) of target antibiotics. Predictive values based the empirical predicted models in this study and literature are shown in Table 4-2. Compared to previously reported empirical models (Stevens-Garmon et al., 2011; Hyland et al., 2012; Lakshminarasimman et al., 2018), the predicted model in this study (*a*=0.63, *b*=1.15) generally shows good prediction for most compounds with Root mean squared error (RMSE) of 0.47 (lower value means better prediction). The model by Stevens-Garmon et al. (2011) (*a*=0.6, *b*=0.69) shows good prediction on amoxicillin (RMSE=0.17), ampicillin (RMSE=0.31) and piperacillin (RMSE=0.29). Hyland et al. (2012) applied f_{oc}=0.44 in the their predictive model, which shows good prediction for amoxicillin (RMSE=0.29), ampicillin (RMSE=0.27), clarithromycin (RMSE=0.25) and azithromycin (RMSE=0.30). Lakshminarasimman et

al. (2018) proposed the model of log K_d= 0.53 log K_{ow} + 1.18 to directly predict the sorption distribution coefficients based on the log K_{ow} values, which shows very good prediction on cefazolin (RMSE=0.29), clarithromycin (RMSE=0.13), azithromycin (RMSE=0.17), sulfamethoxazole (RMSE=0.23) and trimethoprim (RMSE=0.28). However, the sorption distribution coefficients of levofloxacin were underestimated in all predictive models based on the log K_{ow} values. According the introduction in 2.4.1, K_{ow} value represents the hydrophobicity of compounds, while the adsorption of quinolone antibiotics (including levofloxacin) could not only be attibuted to hydrophobic interactions, electrostatic interaction also plays an important role. It is difficult to build the predictive model including the contribution of electrasanic interaction, then the experimental data of levofloxacin would be applied in the further estimation.

Table 4-2 Comparison on measured sorption distribution coefficients with predicted values using the empirical model

	log K_{ow}	Measured log K_d*	Predicted log K_d			
			This study	Stevens-Garmon et al. (2011)	Hyland et al. (2012)	Lakshminarasimman et al. (2018)
Amoxicillin	0.87	1.00±0.20	1.42	0.94	0.80	1.64
Ampicillin	1.35	0.97±0.17	1.72	1.23	1.18	1.90
Piperacillin	0.30	0.83±0.10	1.06	0.60	0.35	1.34
Cefazolin	-0.58	0.66±0.17	0.51	0.07	-0.34	0.87
Clarithromycin	3.16	2.74±0.13	2.86	2.31	2.61	2.85
Azithromycin	3.03	2.65±0.11	2.78	2.23	2.51	2.79
Levofloxacin	2.10	3.00±0.34	2.20	1.68	1.77	2.29
Sulfamethoxazole	0.89	1.67±0.24	1.43	0.95	0.82	1.65
Trimethoprim	0.91	1.91±0.12	1.45	0.96	0.83	1.66
Root mean squared error (RMSE)			0.47	0.71	0.77	0.52

*: average measured log K_d value in all conditions ± standard deviation
Correlation in this study: log K_{oc}= 0.63 log K_{ow} + 1.15, $K_d = f_{oc}K_{oc}$, f_{oc}=0.531;
Stevens-Garmon et al. (2011): log K_{oc}= 0.6 log K_{ow} + 0.69, $K_d = f_{oc}K_{oc}$;
Hyland et al. (2012): log K_{oc}= 0.79 log K_{ow} + 0.47, $K_d = f_{oc}K_{oc}$, f_{oc}=0.440;
Lakshminarasimman et al. (2018): log K_d= 0.53 log K_{ow} + 1.18.

4.3.3 Biodegradation performances

4.3.3.1. First-order kinetics

The first-order kinetic constants (k_{bio}, $L \cdot gVSS^{-1} \cdot d^{-1}$) were calculated by Equation 4-4 for all batch experiments without inhibitors, and biotransformation rates and half-lives of 9 target antibiotics in activated sludge from two STPs are summarized in Table 4-3. These compounds based on the k_{bio} values were also classified as follows (Suarez et al., 2010):

Hardly biodegradable (k_{bio} <0.5);

Moderately biodegradable ($0.5 \leq k_{bio} < 1$);

Highly biodegradable ($1 \leq k_{bio} < 5$);

Very highly biodegradable ($k_{bio} \geq 5$).

Group 1: β-lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), which were highly ($1 \leq k_{bio} < 5$) or very highly biodegradable ($k_{bio} \geq 5$) under three redox conditions and all sludge sources. In STP A, the biodegradation rate of amoxicillin was highest under anoxic condition (k_{bio} =9.34 L/gVSS-d, r^2=1), the aerobic biodegradation rate (k_{bio} =7.75 L/gVSS-d, r^2=1) was slightly higher than anaerobic rate (k_{bio} =7.10 L/gVSS-d, r^2=1). The aerobic rate of amoxicillin in STP B_CAS sludge (k_{bio} =21.97 L/gVSS-d, r^2=0.99) was the highest under three sludge sources (STP A, STP B_CAS, and STP B_MBR). The biodegradation rates and trends of ampicillin under all sludge sources were similar to amoxicillin: STP B_CAS (k_{bio} =28.68 L/gVSS-d, r^2=0.99) > STP B_MBR (k_{bio} =17.64 L/gVSS-d, r^2=0.99) > STP A_Anoxic (k_{bio} =10.51 L/gVSS-d, r^2=0.99) > STP A_Aerobic (k_{bio} =7.94 L/gVSS-d, r^2=1) > STP A_Anaerobic (k_{bio} =7.75 L/gVSS-d, r^2=1). Among all target compounds, cefazolin had the highest biodegradation rates (k_{bio} =11.42-43.15 L/gVSS-d), and the trends of cefazolin under all sludge sources were similar to amoxicillin and ampicillin. The biodegradation rates of piperacillin under anoxic and anaerobic conditions were the same (k_{bio} =2.95 L/gVSS-d), which were slightly higher than aerobic rate (k_{bio} =2.30 L/gVSS-d, r^2=1). The aerobic rate of piperacillin was highest under STP B_MBR sludge (k_{bio} =7.27 L/gVSS-d, r^2=1), followed by under STP B_CAS sludge (k_{bio} =4.88 L/gVSS-d, r^2=0.99). The relatively high biodegradation rates of these four compounds were resulted from the susceptible β-lactam nucleus, which could be easily cleavage via the hydrolysis (Mayers, 2009). The

hydrolyzed transformation products could be the final products or be further degraded, therefore, the identification of their transformation products and the study of their occurrence the persistent in the STP effluent the environment are essential for the proper risk assessment.

Group 2: clarithromycin, azithromycin and trimethoprim, whose biodegradable ability was dependent on the redox condition and sludge source. Clarithromycin was highly biodegradable in STP B_MBR sludge (k_{bio} =2.90 L/gVSS-d, r^2=0.85) and STP A_Aerobic sludge (k_{bio} =1.20 L/gVSS-d, r^2=0.97), while it was moderately biodegradable under STP B_CAS sludge (k_{bio} =1.20 L/gVSS-d, r^2=0.95) and hardly biodegradable under STP A_Anoxic (k_{bio} =0.26 L/gVSS-d, r^2=0.99) and STP A_Anaerobic condition (k_{bio} =0.24 L/gVSS-d, r^2=0.91). Joss et al. (2006) reported biodegradation rates of clarithromycin by CAS and MBR sludge to be 0.5 L/gTSS-d and 2 L/gTSS-d, respectively, which were slightly lower than those in this study. Park (2016) obtained similar result of clarithromycin by CAS sludge (k_{bio} =0.522 L/gVSS-d) to Joss et al. (2006), while the biodegradation rate by MBR sludge (k_{bio} =0.132 L/gVSS-d) was much lower than this study and Joss et al. (2006). Azithromycin was highly biodegradable by STP B_MBR sludge (k_{bio} =2.40 L/gVSS-d, r^2=0.73), moderately biodegradable under STP A_Aerobic condition (k_{bio} =0.77 L/gVSS-d, r^2=0.90), while it was hardly biodegradable under anoxic condition (k_{bio} =0.05 L/gVSS-d, r^2=0.77) and by STP B_CAS sludge (k_{bio} =0.26 L/gVSS-d, r^2=0.80), and no degradation under anaerobic condition. Joss et al. (2006) reported biodegradation rates of azithromycin by CAS and MBR sludge to be 0.15 L/gTSS-d and 1.3 L/gTSS-d, respectively, at rates also slightly lower than this study. Park (2016) observed much lower biodegradation rates of azithromycin by both MBR and CAS sludge (k_{bio} < 0.1 L/gVSS-d). Both clarithromycin and azithromycin were more biodegradable under aerobic condition than anoxic and anaerobic conditions in this study. The nitrifying activity in aerobic condition could probably be the key factor enhancing the biodegradation of these two compounds. It has been reported that the nitrifying activity has a positively association with the biodegradation ability of some micropollutants due to the wide metabolic activity of nitrifying bacteria (e.g., AOB) (Clara et al., 2005b; Göbel et al., 2007; Park, 2016), which may co-metabolize micropollutants through AMO enzyme, and some

heterotrophic microorganisms were also found to degrade some micropollutants together with AOB (Tran et al., 2013). While the catalytic effect AMO enzyme is highly dependent on the structure of compounds, it could be hindered by the presence of specific functional group, like aromatic rings, amide groups, amine groups, and heterocyclic rings that occur in trimethoprim (Fernandez-Fontaina et al., 2016). This could also explain why the biodegradable abilities of trimethoprim were much higher in anoxic (k_{bio} =1.54 L/gVSS-d, r^2=0.96 in STP A_Anoxic) and anaerobic (k_{bio} =2.16 L/gVSS-d, r^2=0.99 in STP A_Anaerobic) conditions than those in aerobic conditions (k_{bio} =0.12 L/gVSS-d, r^2=0.94 in STP A_Aerobic; k_{bio} =0.05 L/gVSS-d, r^2=0.69 in STP B_MBR; no biodegradation in STP B_CAS). Lakshminarasimman et al. (2018) also reported that the biodegradation rates of trimethoprim in anoxic (k_{bio} =0.19 L/gVSS-d) and anaerobic (k_{bio} =0.24 L/gVSS-d) conditions were slightly higher than that in aerobic condition (k_{bio} =0.14 L/gVSS-d), however, the rates in anoxic and anaerobic conditions were much lower than those in this study. Inyang et al. (2016) observed opposite results that the biodegradation rate of trimethoprim in aerobic condition (k_{bio} =5.04 L/gTSS-d) was much higher than that in anoxic (k_{bio} =0.216 L/gTSS-d) and anaerobic (no biodegradation) conditions.

Group 3: sulfamethoxazole and levofloxacin, which were hardly biodegradable under all redox conditions and sludge sources (k_{bio} <0.5 L/gVSS-d). The biodegradation rates of sulfamethoxazole in STP B (k_{bio} =0.49 L/gVSS-d, r^2=0.90 in STP B_CAS; k_{bio} =0.26 L/gVSS-d, r^2=0.78 in STP B_MBR) were slightly higher than those in STP A (k_{bio} =0.19 L/gVSS-d, r^2=0.79 in STP A_Aerobic; k_{bio} =0.24 L/gVSS-d, r^2=0.64 in STP A_Anoxic; no biodegradation in STP A_Anaerobic). Some previous studies also reported that sulfamethoxazole was hardly biodegradable under all redox conditions and sludge sources (Joss et al., 2006; Lakshminarasimman et al., 2018; Park, 2016; Plósz et al., 2010; Suarez et al., 2010). However, it has been reported that N_4-acetylsulfamethoxazole as a main metabolite of sulfamethoxazole could be excreted from human bodies, accounting for more than 50% of the consumption dose, which was higher than the parent compound-sulfamethoxazole. N_4-acetylsulfamethoxazole could de-conjugate into sulfamethoxazole, resulting in the underestimation of biodegradation rates on sulfamethoxazole (Göbel et al., 2007). There was no biodegradation of

levofloxacin in STP B, while it was slightly biodegraded in STP A (k_{bio} =0.05 L/gVSS-d, r^2=0.34 in STP A_Aerobic; k_{bio} =0.31 L/gVSS-d, r^2=0.75 in STP A_Anoxic; no biodegradation in STP A_Anaerobic). Park (2016) also observed no biodegradation of levofloxacin in both MBR and CAS process.

4.3.3.2. Separately characterization on the AOB co-metabolic kinetics

Comparing the biodegradation results between RUN 1 (No inhibitor) and RUN 3 (ATU), the rate and contribution of AOB co-metabolism could be obtained according to 4.2.4.3, and the results were summarized in Table 4-4 and Figure 4-5. As discussed in 4.3.1, the activity of AOB in activated sludge taking from STP A_Aerobic, STP B_CAS and STP B_MBR were 16.22, 36.16 and 26.29 mg N/(gVSS·d), respectively.

The AOB co-metabolic rates of amoxicillin were 0.37 μg/(L·d) in STP A_Aerobic, 0.35 μg/(L·d) in STP B_CAS, and 1.17 μg/(L·d) in STP B_MBR, respectively. The AOB co-metabolic rates of ampicillin, piperacillin and cefazolin were highest in STP B_CAS [k_{AOB}-ampicillin = 4.22 μg/(L·d), k_{AOB}-piperacillin = 2.54 μg/(L·d), and k_{AOB}-cefazolin = 7.42 μg/(L·d)], followed by those in STP B_MBR [k_{AOB}-ampicillin =0.94 μg/(L·d), k_{AOB}-piperacillin = 0.30 μg/(L·d), and k_{AOB}-cefazolin = 7.33 μg/(L·d)] and STP A_Aerobic [k_{AOB}-ampicillin = 0.54 μg/(L·d), k_{AOB}-piperacillin = 0 μg/(L·d), and k_{AOB}-cefazolin = 4.14 μg/(L·d)]. The AOB co-metabolic rate of ampicillin, piperacillin and cefazolin showed positive relationship with the AOB activity in different sludge sources, correspondingly the contribution of AOB co-metabolism was higher in STP B_CAS sludge for these three compounds. Some previous studies also reported a positive relationship between nitrifying activity and AOB co-metabolic rate of some micropollutants, such as ibuprofen, 17α-ethinylestradiol, erythromycin, roxithromycin and so on (Alvarino et al., 2014; Xu et al., 2016). For these very highly biodegradable antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), the contribution of AOB co-metabolism on the biodegradation of these three compounds were relatively low (<5%). It has also been reported that some highly biodegradable micropollutant (e.g., caffeine and theophylline) could be quickly eliminated no matter with or without the presence of ATU (Park et al., 2017).

Table 4-3 Biotransformation rates of 9 target antibiotics calculated by first-order kinetics in activated sludge from two STPs

	STP A									STP B					
	Aerobic			Anoxic			Anaerobic			CAS			MBR		
	k_{bio}, L/(gVSS·d)	r^2	Half-lives (h)	k_{bio}, L/(gVSS·d)	r^2	Half-lives (h)	k_{bio}, L/(gVSS·d)	r^2	Half-lives (h)	k_{bio}, L/(gVSS·d)	r^2	Half-lives (h)	k_{bio}, L/(gVSS·d)	r^2	Half-lives (h)
Amoxicillin	7.75	1.00	1.1	9.34	1.00	1.0	7.10	1.00	1.3	21.97	0.99	0.6	19.12	0.99	0.7
Ampicillin	7.94	1.00	1.1	10.51	0.99	0.9	7.75	1.00	1.2	28.68	0.99	0.5	17.64	0.99	0.8
Piperacillin	2.30	1.00	3.8	2.95	0.98	3.0	2.95	1.00	3.0	4.88	0.99	2.9	7.27	1.00	1.9
Cefazolin	14.26	1.00	0.6	16.03	0.99	0.6	11.42	0.99	0.8	43.15	1.00	0.3	18.07	0.99	0.8
Clarithromycin	1.20	0.97	7.3	0.26	0.99	33.9	0.24	0.91	37.3	0.83	0.95	16.8	2.90	0.85	4.8
Azithromycin	0.77	0.90	11.3	0.05	0.77	186.3	0	-	-	0.26	0.80	53.3	2.40	0.73	5.8
Levofloxacin	0.05	0.34	181.5	0.31	0.75	28.7	0	-	-	0	-	-	0	-	-
Sulfamethoxazole	0.19	0.79	45.4	0.24	0.64	37.3	0	-	-	0.49	0.90	28.4	0.26	0.78	9.4
Trimethoprim	0.12	0.94	72.6	1.54	0.96	5.8	2.16	0.99	4.1	0	-	-	0.05	0.69	51.7

-: not available

The AOB co-metabolic rates of clarithromycin were 0 in STP A_Aerobic, 0.15 μg/(L·d) in STP B_CAS, and 2.81 μg/(L·d) in STP B_MBR, respectively. The contribution of AOB co-metabolism on clarithromycin accounted for 16.8% in STP B_MBR, which was 2.8% in STP B_CAS and 0 in STP A_Aerobic, respectively. The contribution of AOB co-metabolism showed positively relationship with the biodegradability of clarithromycin in activated sludge taken from different sources. For azithromycin, the AOB co-metabolic rates were 0 in STP A_Aerobic, 0 μg/(L·d) in STP B_CAS, and 4.20 μg/(L·d) in STP B_MBR, respectively. The contribution of AOB co-metabolism of azithromycin was also highest in STP B_MBR sludge (20.0%), while there was no AOB co-metabolism detected for azithromycin in STP A_Aerobic and STP B_CAS sludge. As discussed in 4.3.3.1, the biodegradability of clarithromycin and azithromycin were also highest in STP B_MBR sludge. The possible reason might be the MBR system shows higher microbial diversity than CAS and AAO systems. It has been reported that AOB and heterotrophs could cooperate to breakdown micropollutants (Khunjar et al., 2011; Tran et al., 2009), which could explain why the biodegradation rates of both heterotrophs and AOB were improved in STP B_MBR. Khunjar et al. (2011) also found that the AOB exhibited superior elimination of micropollutants than heterotrophs typically, therefore, the contribution of AOB co-metabolism was improved for clarithromycin and azithromycin in STP B_MBR.

For sulfamethoxazole and trimethoprim, the AOB co-metabolism contributed higher than those for other target compounds. There was only AOB co-metabolism of sulfamethoxazole detected in STP A_Aerobic with the rate of 6.74 μg/(L·d), while the AOB co-metabolic rates were 7.44 μg/(L·d) in STP B_CAS and 4.74 μg/(L·d) in STP B_MBR, with a contribution of 40.2% in STP B_CAS and 66.5% in STP B_MBR, respectively. There was no degradation of trimethoprim detected in STP B_CAS, while there was only AOB co-metabolism detected in STP B_MBR with the rate of 1.87 μg/(L·d). The AOB co-metabolic rate of trimethoprim in STP A_Aerobic was 1.03 μg/(L·d) with a contribution of 33.1%. Kassotaki et al. (2016) found that sulfamethoxazole could be highly biodegraded (86%) during the enriched AOB system due to the higher AOB activity. Trimethoprim was also reported that the elimination efficiency decreased from 70% to 28% when AOB was inhibited by adding ATU (Park

et al., 2017). Faster biodegradation kinetics of sulfamethoxazole and trimethoprim have been measured in the nitrifying reactor compared to those in the presence of AOB inhibitor (Kassotaki et al., 2016; Sathyamoorthy et al., 2013).

4.3.3.3. Selection of biodegradation rate constants for further estimation

The r^2 values were used to evaluate the accuracy of the estimation on the biodegradation rate constants of target antibiotics. For the first-order kinetics estimation, the r^2 values were shown in Table 4-3, and the r^2 values of separately estimation AOB co-metabolic kinetics are given in Table 4-4. For the very highly biodegradable antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), due to the low contribution of AOB co-metabolism, the estimation accuracies of first-order kinetics (r^2 = 0.99-1) were comparable with those of separately estimation on AOB co-metabolic kinetics (r^2 = 0.97-1). For the others, except for the estimation on the biodegradation of sulfamethoxazole in STP A_Aerobic and trimethoprim in STP B_MBR, the r^2 values of first-order kinetics were higher than those of separately estimation on the AOB co-metabolic kinetics. There was only AOB co-metabolism detected for the biodegradation of sulfamethoxazole in STP A_Aerobic and trimethoprim in STP B_MBR, therefore, it was more accurate to separately estimate on AOB co-metabolic kinetics. For the estimation on the first-order kinetics, the experimental data of RUN 1 (no inhibitor) was used, there was only one time prediction; while for the separately estimation on the AOB co-metabolic kinetics, there were two datasets applied for prediction of the rate constants (RUN 1 and RUN 3), which resulted in a decrease in accuracy statistically compared to one-time estimation (first-order kinetics). Separately estimation and characterization on the contribution of AOB co-metabolism could help to better understand the mechanisms of biodegradation of each antibiotic in activated sludge, however, the accuracy was more important during the further estimation on the fate of target compounds in STPs. Therefore, the first-order kinetic constants of target antibiotics would be applied in Chapter V.

Table 4-4 Separately characterization on AOB co-metabolic rates of 9 target antibiotics in activated sludge taken from two target STPs

	STP A_Aerobic			STP B_CAS			STP B_MBR		
	k_{bio}' [L/(gVSS·d)]	k_{AOB} [μg/(L·d)]	r^2	k_{bio}' [L/(gVSS·d)]	k_{AOB} [μg/(L·d)]	r^2	k_{bio}' [L/(gVSS·d)]	k_{AOB} [μg/(L·d)]	r^2
Amoxicillin	7.37	0.37	1	22.70	0.35	0.97	18.04	1.17	0.97
Ampicillin	7.32	0.54	1	16.82	4.22	0.97	15.99	0.94	1
Piperacillin	2.30	0	1	4.93	2.54	0.98	7.04	0.30	0.97
Cefazolin	11.95	4.14	0.99	40.89	7.42	1	16.92	7.33	0.99
Clarithromycin	1.20	0	0.97	0.94	0.15	0.75	2.11	2.81	0.71
Azithomycin	0.77	0	0.90	0.31	0	0.68	1.73	4.20	0.67
Levofloxacin	0.05	0	0.34	0	0	-	0	0	-
Sulfamethoxazole	0	6.74	0.81	0.28	7.44	0.65	0.07	4.73	0.75
Trimethoprim	0.07	1.03	0.80	0	0	-	0	1.87	0.78

-: not available

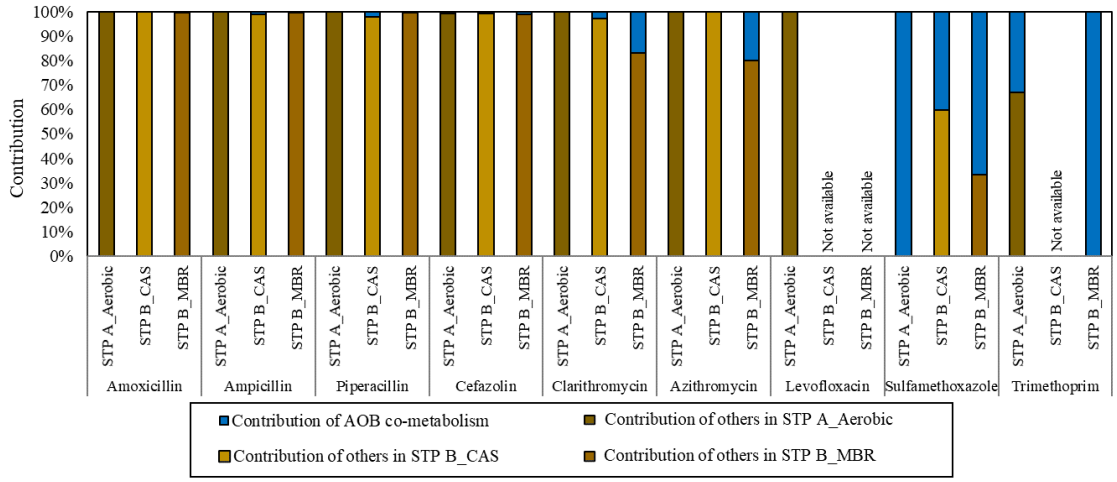


Figure 4-5 Contribution of AOB co-metabolism on the biodegradation of 9 target compounds in the activated sludge taken from two target STPs

4.4 Conclusions

In this Chapter, the sorption distribution coefficients and biodegradation rates were determined for 9 target antibiotics in the activated sludge from three different redox conditions in STP A_AAO, and aerobic condition in STP B_CAS and STP B_MBR. For the sorption distribution coefficients, the empirical predictive model was established and evaluated by the measured data. The biodegradation of target antibiotics was estimated by two kinetics: 1) first-order kinetics; 2) separately characterization on AOB co-metabolic kinetics and heterotroph biodegradation kinetics (first-order kinetics). The findings from this study were as follows:

- ✓ All β-lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), sulfamethoxazole and trimethoprim were classified as lowly sorptive (log K_d < 2). The K_d values of β-lactams (amoxicillin, ampicillin, piperacillin and cefazolin) ranged from 2.4 to 28.4 L/kg (log K_d =0.39-1.45), the average log K_d value (n=5) of these four compounds for all redox conditions and all STPs were 1.00 ± 0.12, 0.98 ± 0.13, 0.83 ± 0.06, and 0.66 ± 0.12, respectively. Clarithromycin and azithromycin were classified as moderately sorptive (2 < log K_d < 3). The K_d values for clarithromycin ranged from 236 to 1315 L/kg (log K_d =2.73 ± 0.13, n=5). Levofloxacin was the only compound classified as highly sorptive (log K_d > 3) in this study, the K_d values ranged from 272 to 6484 L/kg (log K_d =3.00 ± 0.34, n=5).
- ✓ There were high correlations of log K_d values between different redox conditions (R^2 > 0.9), and the highest correlation was between anoxic and anaerobic activated sludge (R^2 = 0.993). Variability of sulfamethoxazole in log K_d values were more than those of other compounds.
- ✓ The predictive model of sorption distribution coefficient in this study was established and would be applied in further estimation in Chapter V: log K_{oc}= 0.63 log K_{ow} + 1.15, $K_d = f_{oc}K_{oc}$, f_{oc}=0.531, which generally shows good prediction for most compounds with RMSE=0.47. However, the sorption distribution coefficients of levofloxacin were underestimated in all predictive models based on the log K_{ow} values, then the experimental data of levofloxacin would be applied in the further estimation.
- ✓ The first-order kinetic constants (k_{bio}, $L \cdot gVSS^{-1} \cdot d^{-1}$) were estimated on the

biodegradation of target antibiotics in the activated sludge from three different redox conditions in STP A_AAO, and aerobic condition in STP B_CAS and STP B_MBR. B-Lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin) were highly ($1 < k_{bio} < 5$) or very highly biodegradable ($k_{bio} > 5$) under three redox conditions and all sludge sources. Sulfamethoxazole and levofloxacin were hardly biodegradable under all redox conditions and sludge sources ($k_{bio} < 0.5$ L/gVSS-d).

- ✓ The biodegradable abilities of clarithromycin, azithromycin and trimethoprim were dependent on the redox condition and sludge source. Both clarithromycin and azithromycin were more biodegradable under aerobic condition than anoxic and anaerobic conditions in this study. While the biodegradable abilities of trimethoprim were much higher in anoxic (k_{bio} =1.54 L/gVSS-d, r^2=0.96 in STP A_Anoxic) and anaerobic (k_{bio} =2.16 L/gVSS-d, r^2=0.99 in STP A_Anaerobic) conditions than those in aerobic conditions (k_{bio} =0.12 L/gVSS-d, r^2=0.94 in STP A_Aerobic; k_{bio} =0.05 L/gVSS-d, r^2=0.69 in STP B_MBR; no biodegradation in STP B_CAS).
- ✓ For the highly and very highly biodegradable antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), the contribution of AOB co-metabolism on the biodegradation of these three compounds were relatively low (<5%). The AOB co-metabolic rate of ampicillin, piperacillin and cefazolin showed positive relationship with the AOB activity in different sludge sources. The contribution of AOB co-metabolism of clarithromycin and azithromycin was highest in STP B_MBR sludge, accounting for 16.8% and 20.0%, respectively. For sulfamethoxazole and trimethoprim, the AOB co-metabolism contributed higher than of other target compounds.
- ✓ Separately estimation and characterization on the contribution of AOB co-metabolism could help to better understand the mechanisms of biodegradation of each antibiotic in activated sludge, however, the estimation accuracy was higher for first-order kinetics due to the one-time estimation. Therefore, the first-order biodegradation rate constants of target antibiotics would be applied for further estimation in Chapter V.

4.5 Reference

Abegglen, C., Joss, A., McArdell, C. S., Fink, G., Schlüsener, M. P., Ternes, T. A., Siegrist, H., 2009. The fate of selected micropollutants in a single-house MBR. *Water Research*, *43*(7), pp.2036-2046.

Alvarino, T., Suarez, S., Lema, J. M., Omil, F., 2014. Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. *Journal of Hazardous Materials*, *278*, pp.506-513.

Apha, A., 2007. WEF (2005) Standard methods for the examination of water and wastewater. *American Public Health Association, American Water Works Association, and Water Environment Federation*.

Blair, B., Nikolaus, A., Hedman, C., Klaper, R., Grundl, T., 2015. Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. *Chemosphere*, *134*, pp.395-401.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O., Kroiss, H., 2005b. The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Research*, *39*(1), pp.97-106.

Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., Kroiss, H., 2005a. Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. *Water Research*, *39*(19), pp.4797-4807.

Fernandez-Fontaina, E., Gomes, I. B., Aga, D. S., Omil, F., Lema, J. M., Carballa, M., 2016. Biotransformation of pharmaceuticals under nitrification, nitratation and heterotrophic conditions. *Science of the Total Environment*, *541*, pp.1439-1447.

Göbel, A., McArdell, C. S., Joss, A., Siegrist, H., Giger, W., 2007. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of the Total Environment*, *372*(2-3), pp.361-371.

Göbel, A., Thomsen, A., McArdell, C. S., Joss, A., Giger, W., 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. *Environmental Science & Technology*, *39*(11), pp.3981-3989.

Hyland, K. C., Dickenson, E. R., Drewes, J. E., Higgins, C. P., 2012. Sorption of ionized and neutral emerging trace organic compounds onto activated sludge from different wastewater treatment configurations. *Water Research*, *46*(6), pp.1958-1968.

Inyang, M., Flowers, R., McAvoy, D., Dickenson, E., 2016. Biotransformation of trace organic compounds by activated sludge from a biological nutrient removal treatment system. *Bioresource Technology*, *216*, pp.778-784.

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. *Water Research*, *40*(8), pp.1686-1696.

Kassotaki, E., Buttiglieri, G., Ferrando-Climent, L., Rodriguez-Roda, I., Pijuan, M., 2016. Enhanced sulfamethoxazole degradation through ammonia oxidizing bacteria co-metabolism and fate of transformation products. *Water Research*, *94*, pp.111-119.
Kassotaki, E., Buttiglieri, G., Ferrando-Climent, L., Rodriguez-Roda, I., Pijuan, M., 2016. Enhanced sulfamethoxazole degradation through ammonia oxidizing bacteria co-metabolism and fate of transformation products. *Water Research*, *94*, pp.111-119.
Kazama S., 2017. Removal characteristics and mechanisms of pharmaceuticals in membrane bioreactor. (Master's Thesis) Graduate School of Engineering, Kyoto University, Japan.

Khunjar, W. O., Mackintosh, S. A., Skotnicka-Pitak, J., Baik, S., Aga, D. S., Love, N. G., 2011. Elucidating the relative roles of ammonia oxidizing and heterotrophic bacteria during the biotransformation of 17α-ethinylestradiol and trimethoprim. *Environmental Science & Technology*, *45*(8), pp.3605-3612.

Kumwimba, M. N., Meng, F., 2019. Roles of ammonia-oxidizing bacteria in improving metabolism and cometabolism of trace organic chemicals in biological wastewater treatment processes: a review. *Science of the Total Environment*, *659*, pp.419-441.

Lakshminarasimman, N., Quiñones, O., Vanderford, B. J., Campo-Moreno, P., Dickenson, E. V., McAvoy, D. C., 2018. Biotransformation and sorption of trace organic compounds in biological nutrient removal treatment systems. *Science of the Total Environment*, *640*, pp.62-72.

Mayers, D.L. ed., 2009. *Antimicrobial drug resistance* (pp. 803-824). New York: Humana press.

Mazioti, A. A., Stasinakis, A. S., Gatidou, G., Thomaidis, N. S., Andersen, H. R., 2015. Sorption and biodegradation of selected benzotriazoles and hydroxybenzothiazole in activated sludge and estimation of their fate during wastewater treatment. *Chemosphere*, *131*, pp.117-123.

McCarty, P.L., 1974. Anaerobic process, In Birmingham Short Course on Design Aspects of Biological Treatment, Int'l Assoc. of Water Pollution Research, Birmingham, England.

Namkung, E., Rittmann, B. E., 1987. Estimating volatile organic compound emissions from publicly owned treatment works. *Journal (Water Pollution Control Federation)*, pp.670-678.

Narumiya, M., 2011. Simultaneous quantification of PPCPs and their fate in municipal wastewater treatment plants. (Master's Thesis) Graduate School of Engineering, Kyoto University, Japan.

Park J., 2016. Removal characteristics and predictive model of pharmaceuticals and personal care products (PPCPs) in membrane bioreactor (MBR) process. Doctoral Thesis) Graduate School of Engineering, Kyoto University, Japan.

Park, J., Yamashita, N., Wu, G., Tanaka, H., 2017. Removal of pharmaceuticals and personal care products by ammonia oxidizing bacteria acclimated in a membrane bioreactor: Contributions of cometabolism and endogenous respiration. *Science of the Total Environment*, *605*, pp.18-25.

Petrie, B., McAdam, E. J., Lester, J. N., Cartmell, E., 2014. Assessing potential modifications to the activated sludge process to improve simultaneous removal of a diverse range of micropollutants. *Water Research*, *62*, pp.180-192.

Plósz, B. G., Leknes, H., Thomas, K. V., 2010. Impacts of competitive inhibition, parent compound formation and partitioning behavior on the removal of antibiotics in municipal wastewater treatment. *Environmental Science & Technology*, *44*(2), pp.734-742.

Plósz, B. G., Leknes, H., Thomas, K. V., 2010. Impacts of competitive inhibition, parent compound formation and partitioning behavior on the removal of antibiotics in municipal wastewater treatment. *Environmental Science & Technology*, *44*(2), pp.734-742.

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

Sathyamoorthy, S., Chandran, K., Ramsburg, C. A., 2013. Biodegradation and cometabolic modeling of selected beta blockers during ammonia oxidation. *Environmental Science & Technology*, *47*(22), pp.12835-12843.

Smolders, G. J. F., Van der Meij, J., Van Loosdrecht, M. C. M., Heijnen, J. J., 1994. Model of the anaerobic metabolism of the biological phosphorus removal process: stoichiometry and pH influence. *Biotechnology and Bioengineering*, *43*(6), pp.461-470.

Stevens-Garmon, J., Drewes, J. E., Khan, S. J., McDonald, J. A., Dickenson, E. R.，2011. Sorption of emerging trace organic compounds onto wastewater sludge solids. *Water Research*, *45*(11), pp.3417-3426.

Suarez, S., Lema, J. M., Omil, F., 2010. Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. *Water Research*, *44*(10), pp.3214-3224.

Tran, N. H., Urase, T., Kusakabe, O., 2009. The characteristics of enriched nitrifier culture in the degradation of selected pharmaceutically active compounds. *Journal of Hazardous Materials*, *171*(1-3), pp.1051-1057.

Tran, N. H., Urase, T., Ngo, H. H., Hu, J., Ong, S. L., 2013. Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. *Bioresource Technology*, *146*, pp.721-731.

Vuono, D. C., Benecke, J., Henkel, J., Navidi, W. C., Cath, T. Y., Munakata-Marr, J., Spear, J.R., Drewes, J. E., 2015. Disturbance and temporal partitioning of the activated sludge metacommunity. *The ISME Journal*, *9*(2), pp.425-435.

Xu, K., Harper Jr, W. F., Zhao, D., 2008. 17α-Ethinylestradiol sorption to activated sludge biomass: Thermodynamic properties and reaction mechanisms. *Water Research*, *42*(12), pp.3146-3152.

Xu, Y., Yuan, Z., Ni, B. J., 2016. Biotransformation of pharmaceuticals by ammonia oxidizing bacteria in wastewater treatment processes. *Science of the Total Environment*, *566*, pp.796-805.

Xue, W., Wu, C., Xiao, K., Huang, X., Zhou, H., Tsuno, H., Tanaka, H., 2010. Elimination and fate of selected micro-organic pollutants in a full-scale anaerobic/anoxic/aerobic process combined with membrane bioreactor for municipal wastewater reclamation. *Water Research*, *44*(20), pp.5999-6010.

Chapter V

Estimation on the fate of antibiotics in sewage treatment plants (STPs) through plant-wide modelling

5.1 Introduction

Since the discharges of STPs have been identified as the main source for human-used antibiotics entered into the aquatic environment (Oberoi et al., 2019), the study on the fate of antibiotics in the STPs is required for the risk assessment and the identification of strategies to improve their removal as well. Numerous studies investigated the fate of antibiotics in the STPs (Le-Minh et al., 2010; Wang et al., 2020; Zhang and Li, 2011), however, as discussed in 3.1, it is essential to develop proper predictive models to estimate the fate of antibiotics in the STPs.

In a stream of STPs, the total concentration of antibiotics is expressed as the sum of its soluble and sorbed concentration. If the calculation on the mass balance of antibiotics just based on the concentration without considering the balance of flow rate and SS loading, it would result in the biased and unreliable data on the removal efficiency (Ort et al., 2010). Hence, it is necessary to make corrections on the balance of flow rate and SS loading and calculate the fate of antibiotics based on the loading of each unit. Some researcher proposed the models for individual units based on the mass balance concept, such as primary treatment unit (Carballa et al., 2008), activated sludge unit (Alvarino et al., 2014; Guo and Vanrolleghem, 2014), sludge thickening and dewatering unit (Gernaey et al., 2014), anaerobic sludge digester unit (Taboada-Santos et al., 2019) and so on. It is essential to applied the plant-wide simulation through the integration of multiple-unit models to estimate the fate of antibiotics in the STPs. The aim of this study is to evaluate the discharge of antibiotics to the aquatic environment, thus the models for the water stream (primary and secondary treatment units) would be applied.

In this Chapter, the integration of the primary and secondary treatment models was applied to estimate the fate of antibiotics in the STPs. Meanwhile, the fate of antibiotics in two target STPs were determined, and the mass balance-based removal efficiency of antibiotics was calculated to evaluate the accuracy of the plant-wide simulation. Finally, the discharge of antibiotics from the target STPs were obtained, and the strategies to improve the removal of antibiotics in the STPs were identified by comparing the removal performances of antibiotics in three activated sludge systems.

5.2 Materials and Methods

5.2.1 Sampling

Sampling was conducted at the two target STPs from May, 2019 to March, 2020 (n=6), the process flows and sampling points of these two STPs are shown in Figure 5-1, the basic information on the process, flow rate, service population, and location are given in Table 3-2 and 4.2.1. One liter of sample was collected in 1L-glass bottle covered by aluminum foil for each sampling point, 1 g of ascorbic acid was added and mixed immediately in each bottle after sampling. All samples were stored at 4 ℃ and processed within 24 h.

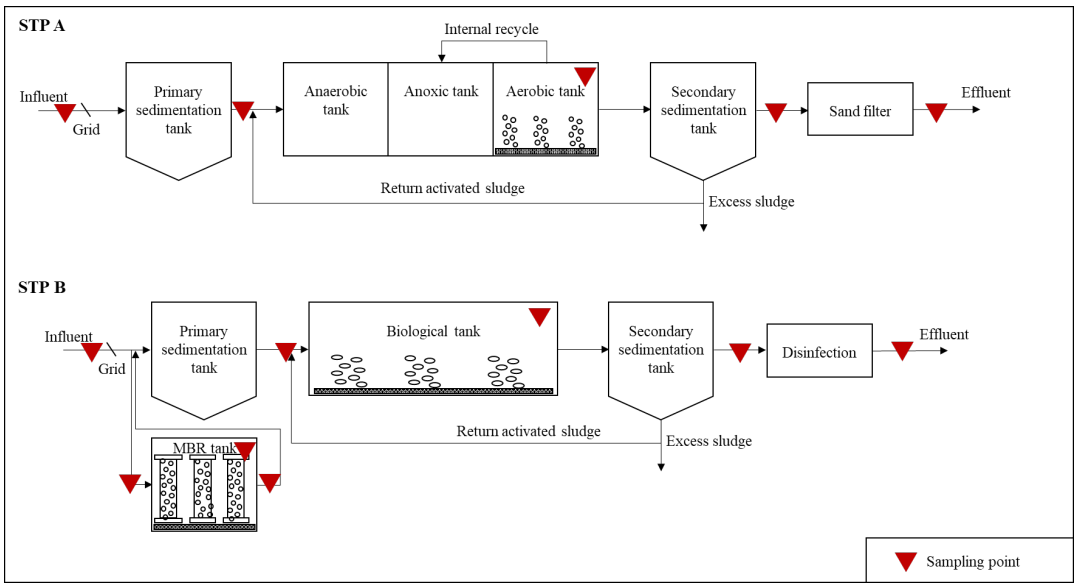


Figure 5-1 Process diagram and sampling points of two target STPs

5.2.2 Analytical methods

The 9 target antibiotics (amoxicillin, ampicillin, piperacillin, cefazolin, clarithromycin, azithromycin, levofloxacin, sulfamethoxazole and trimethoprim) were analyzed in both liquid and solid phases in the samples of influent, effluent of primary sedimentation tank and sludge, while these compounds were only detected in the liquid phase of secondary effluent and final effluent due to the low SS concentration. The pretreatment procedures of liquid phase in all samples are described in 3.2.3. For the solid phase extraction, 1g of wet sludge for sludge samples and the residue at the filter after filtration of 100-ml sample through a 1-μm glass fiber filter for influent and effluent of primary sedimentation were prepared to extract the solid phase, the extraction procedures are also described in 3.2.3.

The target compounds are separate and detected by a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) device equipped with an ACQUITY BEH C_{18} (octadecylsilica-based) column (1.7 μm, 2.1 mm × 100 mm, Waters), as described in 3.2.3.

5.2.3 Calculation of mass balance

The removal of antibiotics in the STPs mainly occur through biodegradation by microorganism in the activated sludge and adsorption onto the suspended solid and discharged as primary sludge and excess sludge. Therefore, the mass balance of antibiotics in the STP was based on the following equation (Narumiya, 2011):

$$F_{inf} = F_{eff} + F_{bio} + F_{prim} + F_{exc} + F_D \qquad \text{(Equation 5-1)}$$

Where, F_{inf} (g/d) is the load of antibiotics in the sum of liquid and solid phases of influent;

F_{eff} (g/d) is the load of antibiotics in the liquid phase of effluent;

F_{prim} (g/d) is the discharge load of antibiotics in the sum of liquid and solid phases of primary sludge;

F_{exc} (g/d) is the discharge load of antibiotics in the sum of liquid and solid phases of excess sludge;

F_{bio} (g/d) is the load of antibiotics biodegraded by the microorganisms in the activated sludge, which could be calculated by Equation 5-2:

$$F_{bio} = F_{bio\_inf} - F_{sec\_eff} - F_{exc} \quad \text{(Equation 5-2)}$$

Where, F_{bio_inf} (g/d) is the load of antibiotics in the sum of liquid and solid phases of influent of biological tank;

F_{sec_eff} (g/d) is the load of antibiotics in the liquid phase of secondary effluent.

F_D (g/d) is the load of antibiotics removed by final treatment, e.g., sand filter or disinfection, which can be calculated by following equation:

$$F_D = F_{sec\_eff} - F_{eff} \quad \text{(Equation 5-3)}$$

Based on the calculation of mass balance, the removal of antibiotics by the whole system (R_{all}), adsorption in primary sludge (T_{prim}), biodegradation in the activated sludge ($BioD$), adsorption in excess sludge (T_{exc}), and sand filter or disinfection (D) were calculated by the equations as follows:

$$R_{all}\ (100\%) = \frac{F_{inf} - F_{eff}}{F_{inf}} \times 100 \quad \text{(Equation 5-4)}$$

$$T_{prim}\ (100\%) = \frac{F_{prim}}{F_{inf}} \times 100 \quad \text{(Equation 5-5)}$$

$$BioD\ (100\%) = \frac{F_{bio\_inf} - F_{sec\_eff} - F_{exc}}{F_{inf}} \times 100 \quad \text{(Equation 5-6)}$$

$$T_{exc}\ (100\%) = \frac{F_{exc}}{F_{inf}} \times 100 \quad \text{(Equation 5-7)}$$

$$D\ (100\%) = \frac{F_D}{F_{inf}} \times 100 \quad \text{(Equation 5-8)}$$

$$R_{all} = T_{prim} + BioD + T_{exc} + D \quad \text{(Equation 5-9)}$$

5.2.4 Estimation models

5.2.4.1. Raw sewage

In a stream in the STP, raw sewage as well, the total concentration of antibiotics (C_t, mg/m^3) is expressed as the sum of their concentration in liquid phase (C_{aq}, mg/m^3) and solid phase (C_S, mg/m^3) (Equation 5-10).

$$C_t = C_{aq} + C_S \quad \text{(Equation 5-10)}$$

The fraction of antibiotics sorbed onto the suspended solids (SS) is represented as the sorption distribution coefficient (K_d), described by Equation 4-1. Hence, C_t can be obtained by combining Equation 5-10 and Equation 4-1:

$$C_t = C_{aq} + C_{aq} \cdot K_d \cdot SS \qquad \text{(Equation 5-11)}$$

5.2.4.2. Primary sedimentation

The primary sedimentation was modelled on the basis of gravity settling principle, which is described by Takács et al. (1991). The fate of antibiotics in the primary sedimentation was modelled by the assumption that there is no biodegradation occurred and the removal is attributed to the adsorption to the primary sludge (Taboada-Santos et al., 2020). Therefore, the following mass balance can be established:

$$F_{prim\_eff} = F_{inf} - F_{prim} \qquad \text{(Equation 5-12)}$$

Where, F_{prim_eff} is the loading of antibiotics in the primary effluent, $F_{prim\_eff} = Q_{prim\_eff} \times C_{t\_prim\_eff}$, $F_{inf} = Q_{inf} \times C_{t\_inf}$, and $F_{prim} = Q_{prim\_sludge} \times C_{t\_prim\_sludge}$, Q_{inf}, Q_{prim_eff} and Q_{prim_sludge} is the flow rate (m^3/d) of influent, primary effluent and discharged primary sludge, respectively, C_{t_inf}, $C_{t\_prim\_eff}$ and $C_{t\_prim\_sludge}$ is the relative concentration (mg/m^3) of antibiotic, which can be calculated by Equation 5-11. Assuming that the antibiotics concentration in the liquid phase of primary effluent and in the primary sludge is exactly the same, and the sorption distribution coefficient of antibiotics in the influent, primary sludge and primary effluent is also exactly same, the antibiotics concentration in the liquid phase of primary effluent ($C_{aq\_prim\_eff}$, mg/m^3) can be calculated by the equation as follows:

$$C_{aq\_prim\_eff} = \frac{Q_{inf} \cdot C_{t\_inf}}{Q_{prim\_eff} \cdot \left(1 + K_{d\_prim} \cdot SS_{prim\_eff}\right) + Q_{prim\_sludge} \cdot \left(1 + K_{d\_prim} \cdot SS_{prim\_sludge}\right)}$$

(Equation 5-13)

Where, K_{d_prim} (m^3/kg) is the sorption distribution coefficient of antibiotics in primary sludge, SS_{prim_eff} and SS_{prim_sludge} is the suspended solid concentration (kg/m^3) in primary effluent and primary sludge, respectively.

5.2.4.3. Biological treatment

The biological reactor was considered as the steady-state, and elimination of antibiotics occurred via biodegradation and discharge of excess sludge. The mass balance for all biological reactors can be established as Equation 5-2, and $C_{t\_bio\_inf}$ and $C_{t\_prim\_eff}$ is exactly same. Therefore, the following equation can be obtained:

$$F_{bio} = Q_{bio\_inf} \cdot C_{t\_prim\_eff} - Q_{sec\_eff} \cdot C_{t\_sec\_eff} - Q_{exc} \cdot C_{t_{exc}} \qquad \text{(Equation 5-14)}$$

Where Q_{bio_inf}, Q_{sec_eff} and Q_{exc} are the flow rate (m^3/d) of biological influent, secondary effluent and discharged excess sludge, respectively, $C_{t\_bio\_inf}$, $C_{t\_sec\_eff}$ and C_{t_exc} are the relative concentration (mg/m^3) of antibiotics.

As discussed in 4.3.3, the biodegradation of antibiotics in activated sludge was assumed as first-order kinetics, and the antibiotics concentration in the liquid phase of secondary effluent and in the activated sludge is exactly the same. hence, the biodegraded load of antibiotics in the biological reactor can be expressed by Equation 5-15:

$$F_{bio}=k_{bio} \cdot VSS \cdot C_{t\_sec\_eff} \cdot V \qquad \text{(Equation 5-15)}$$

Where, k_{bio} ($m^3 \cdot kgVSS^{-1} \cdot d^{-1}$) is the first-order biodegradation kinetic constant of antibiotics, V is the volume of reactor (m^3). Assuming that the antibiotics concentration in the liquid phase and solid phase in the activated sludge, excess sludge and secondary effluent are in equilibrium (Taboada-Santos et al., 2020), and the antibiotics concentration in the liquid phase of secondary effluent ($C_{aq\_sec\_eff}$, mg/m^3) can be calculated by the following equation:

$$C_{aq\_sec\_eff} = \frac{Q_{bio\_inf} \cdot C_{t\_prim\_eff}}{Q_{sec\_eff} \cdot (1+K_{d\_AS} \cdot SS_{sec\_eff}) + Q_{exc} \cdot (1+K_{d\_AS} \cdot SS_{exc}) + k_{bio} \cdot VSS \cdot V \cdot (1+K_{d\_AS} \cdot SS_{sec\_eff})}$$

(Equation 5-16)

Where, K_{d_AS} (m^3/kg) is the sorption distribution coefficient of antibiotics in activated sludge, SS_{sec_eff} and SS_{exc} is the suspended solid concentration (kg/m^3) in secondary effluent and excess sludge, respectively.

5.2.5 Input data of the models

5.2.5.1. The concentration of antibiotics in the sewage influent

To further compare the evaluation on predictive modes for PEC_{inf} and PEC_{eff} of STPs, the average concentration of measured antibiotics in the influent of each STP were applied in the fate estimation in the target STPs, and the data is summarized in Table 5-1.

Table 5-1 The input concentration of antibiotics in the influent of two target STPs

	Input concentration in the sewage influent (mg/m^3)		
	STP A	STP B_CAS	STP B_MBR
Amoxicillin	0.090	0.317	0.242
Ampicillin	0.060	0.235	0.163
Piperacillin	0.431	0.507	0.440
Cefazolin	0.076	0.298	0.252
Clarithromycin	0.580	0.581	0.491
Azithromycin	0.176	0.237	0.305
Levofloxacin	0.570	1.137	0.958
Sulfamethoxazole	0.386	0.233	0.252
Trimethoprim	0.146	0.126	0.107

5.2.5.2. Flow rate, SS concentration and volume of biological reactor

The volume of biological reactor was also required to calculate the biodegraded load of antibiotics (Equation 5-15), and the specification of the biological tank in the target STPs is shown in Table 5-2. To calculate the mass balance of antibiotics in the stream of target STPs, as the calculating equations shown above, the flow rate and SS concentration of each unit in the target STPs are required, which is summarized in Table 5-3.

Table 5-2 The specification of biological reactor in two target STPs

	Volume	No.
STP A	Anaerobic tank 580 m^3 (W9.4×L9.5×H6.5)	1
	Anoxic tank 885 m^3 (W9.4×L14.0×H6.5)	1
	Anoxic tank 715 m^3 (W9.4×L11.7×H6.5)	1
	Aerobic tank 541 m^3 (W9.4×L8.85×H6.5)	4
	Aerobic tank 608 m^3 (W9.4×L9.95×H6.5)	2
STP B_CAS	5,061 m^3 (W7.4×L76.0×H4.5×2)	4
	15,200 m^3 (W10.0×L76.0×H10.0×2)	3
STP B_MBR	44 m^3 (W2.3×L6.4×H3.0)	2

Table 5-3 The flow rate and SS concentration in the stream of two target STPs

		STP A	STP B	
			CAS	MBR
Flow rate (Q, m^3/d)	Q_{inf}	71,440	141,500	710
	Q_{prim_eff}	70,672	137,300	-
	Q_{prim_sludge}	768	4200	-
	Q_{bio_inf}	70,672	136,240	-
	Q_{sec_eff}	69,989	114,218	-
	Q_{exc}	683	2,100	6.71
	Q_{eff}	69,989	122,700	703
SS concentration (kg/m^3)	SS_{inf}	0.200	0.152	0.300
	SS_{prim_eff}	0.067	0.030	-
	SS_{prim_sludge}	11.863	3.112	-
	$MLSS$	2.500	1.312	9.393
	$MLVSS$	1.945	1.109	7.760
	SS_{sec_eff}	0.002	0.001	-
	SS_{exc}	7.27	3.564	9.393
	SS_{eff}	N.D.	N.D.	N.D.

-: no data

N.D.: not detected.

5.2.5.3. Sorption distribution coefficients (K_d) in different sludge

As the results in 4.3.2.5, the empirical predictive model for K_d based on the octanol-water partitioning coefficient (K_{ow}) has been proposed as log K_{oc}= 0.63 log K_{ow} + 1.15, $K_d = f_{oc}K_{oc}$. As mentioned in 4.2.2.1, f_{oc} is the fraction of organic carbon present on the sludge (kg_{oc}/kg_{SS}), based on the composition of primary sedimentation sludge and activated sludge (McCarty, 1974; Namkung and Rittmann, 1987), f_{oc_prim}=0.597, f_{oc_act} and f_{oc_secd} =0.531; and K_{oc} is the organic-carbon distribution coefficient (L/kg_{oc}). The estimated K_d values of target antibiotics in the primary sludge and activated sludge would be applied in the fate estimation, which was summarized in Table 5-4. Since the electrostatic interactions rather than hydrophobic forces play a significant role for the adsorption mechanism of quinolones (Ferreira et al., 2016; Vasudevan et al., 2009), and the K_d of levofloxacin was underestimated, the experimental data in this study would be applied for its further estimation.

Table 5-4 The K_d (m^3/kg) value of target antibiotics in different sludge for the plant-wide estimation

	K_d (m^3/kg)	
	Primary sludge	Activated and excess sludge
Amoxicillin	0.030	0.026
Ampicillin	0.060	0.053
Piperacillin	0.013	0.012
Cefazolin	0.004	0.003
Clarithromycin	0.826	0.734
Azithromycin	0.684	0.608
Levofloxacin	0.446	1.392
Sulfamethoxazole	0.031	0.027
Trimethoprim	0.032	0.028

5.2.5.4. Biodegradation rate constant (k_{bio}) in different biological reactors

Since the first-order kinetics of antibiotics were evaluated to be more accurate in the estimation of biodegradation in 4.3.3, the pseudo-first order biodegradation rate constant of antibiotics (Table 4-3) in the activated sludge of different biological reactors in target STPs were applied to estimate the biodegraded load of antibiotics (Equation 5-15).

5.3 Results and Discussions

5.3.1 Results of water quality

The water qualities in the two target STPs are summarized in Table 5-5. The BOD_5 and TSS concentration in the influent of STP A are higher than those of STP B, while the concentration of COD_{Cr}, T-N and T-P are comparable. For BOD_5, COD_{Cr} and TSS, the removal performances in the two STPs are relatively high, with a removal efficiency of above 99% for BOD_5, above 91% for COD_{Cr}, and 100% for TSS. However, since the A/A/O process (STP A) have separate anaerobic and anoxic tank, the removal efficiency of T-N (81.3±0.9%, n=12) and T-P (98.7±0.5%, n=12) in STP A are higher than those in STP B. The variation of water quality and removal performance in the two STP indicates that the microbial community structure and the activity of functional

microorganisms varied in the activated sludge of the two STPs, which would also influence the removal performance of antibiotics.

Table 5-5 Water quality of two target STPs (n=12), each blank shows the average value and standard deviation

Parameter	STP A			STP B		
	Influent (mg/L)	Effluent (mg/L)	Removal efficiency	Influent (mg/L)	Effluent (mg/L)	Removal efficiency
BOD_5	168±6	0.7±0.1	99.7±0.2%	128±39	1.7±0.3	99.4±0.8%
COD_{Cr}	96.1±3.2	5.3±0.2	94.4±0.1%	90.0±19.0	7.4±0.6	91.5±1.6%
TSS	188±5	N.D.	100%	152±59	N.D.	100%
T-N	29.4±1.0	5.1±0.3	81.3±0.9%	30.2±5.0	13.2±0.7	55.2±5.6%
T-P	3.45±0.16	0.06±0.01	98.7±0.5%	3.12±0.82	1.67±0.80	45.1±29.1%

N.D.: not detected.

5.3.2 Fate and removal of measured antibiotics in water line

5.3.2.1. Fate of measured antibiotics in water line

The concentrations of target antibiotics in the influent, primary effluent, secondary effluent and effluent of STP A and STP B are shown in Table 5-6 and Table 5-7, respectively. All the target antibiotics can be detected in influent and primary effluent. The concentrations of amoxicillin, ampicillin and cefazolin in secondary of effluent and effluent were below the limits of detection (LOD) in the two STPs. In STP A, the highest average concentration of target antibiotics detected in the effluent was for sulfamethoxazole (263 ng/L), and the concentrations of other compounds detected in the effluent were all lower than 100 ng/L. In STP B, the concentrations of all target compounds in MBR-effluent were lower than those in CAS-effluent. Except for β-lactams (amoxicillin, ampicillin, piperacillin and cefazolin), the concentrations of other target antibiotics observed in CAS-effluent were higher than 100 ng/L, with the highest average concentration of 472 ng/L for levofloxacin. In MBR-effluent, the highest detected average concentration was also for levofloxacin (397 ng/L), while the concentrations of other compounds were below 100 ng/L.

Table 5-6 Concentrations (average±standard deviation, ng/L, n=6) of target antibiotics in the water line (influent, primary effluent, secondary effluent and effluent) of STP A

Compound	Concentrations (ng/L) in STP A			
	Primary influent	Primary effluent	Secondary effluent	Effluent
Amoxicillin	90±14	76±11	<LOD	<LOD
Ampicillin	60±21	47±20	<LOD	<LOD
Piperacillin	431±288	449±274	41±21	28±11
Cefazolin	76±28	79±31	<LOD	<LOD
Clarithromycin	580±148	541±136	110±31	83±22
Azithromycin	176±21	185±61	20±4	16±2
Levofloxacin	570±143	410±142	161±36	60±8
Sulfamethoxazole	386±251	249±152	214±122	191±99
Trimethoprim	146±33	142±29	86±26	63±14

<LOD: not detected at the concentrations above limits of detection.

Table 5-7 Concentrations (average±standard deviation, ng/L, n=6) of target antibiotics in the water line (influent, primary effluent, secondary effluent and effluent) of STP B

Compound	Concentrations (ng/L) in STP B					
	CAS				MBR	
	Influent	Primary effluent	Secondary effluent	Effluent	Influent	Effluent
Amoxicillin	317±77	249±63	<LOD	<LOD	242±93	<LOD
Ampicillin	235±108	197±94	<LOD	<LOD	163±86	<LOD
Piperacillin	507±263	442±210	90±32	65±16	440±242	20±9
Cefazolin	298±159	251±128	<LOD	<LOD	252±139	<LOD
Clarithromycin	581±132	516±136	367±23	359±38	491±123	75±41
Azithromycin	237±67	206±63	167±12	171±23	305±118	33±22
Levofloxacin	1137±243	700±133	493±105	472±112	958±301	397±81
Sulfamethoxazole	233±71	204±69	212±32	209±179	252±135	84±28
Trimethoprim	126±27	117±26	112±18	101±18	107±24	17±12

<LOD: not detected at the concentrations above limits of detection.

5.3.2.2. Balance of flow rate and SS load

Since the calculation on mass balance of antibiotics involved the flow rate and SS concentration, it is essential to confirm that the balance of these two parameters is

matched in the treatment system. As the mass balance calculating equations shown in 5.2.3, there are three parts of balance we should consider: 1) primary sedimentation; 2) secondary sedimentation; and 3) the entire system. The balance of before and after primary sedimentation, secondary sedimentation and the entire system were calculated by Equation 5-17, 5-18 and 5-19 (Narumiya, 2011), respectively:

The balance of primary sedimentation

$$= \frac{\text{Effluent of primary sedimentation+Discharge of primary sludge}}{\text{Influent of primary sedimentation}}$$

(Equation 5-17)

The balance of secondary sedimentation

$$= \frac{\text{Effluent of secondary sedimentation+Recycled sludge+Discharge of excess sludge}}{\text{Influent of secondary sedimentation}}$$

(Equation 5-18)

The balance of the entire system

$$= \frac{\text{Effluent+Discharge of primary sludge+Discharge of excess sludge}}{\text{Influent}}$$

(Equation 5-19)

The balance of both flow rate and SS loading should be calculated for primary and secondary sedimentation, while only the balance of flow rate should be considered for the entire system due to the grow of activated sludge. If the values obtained by Equation 5-17, 5-18 and 5-19 are closed to 1 (1±0.1) (Narumiya, 2011), the balance can be considered to be matched. After calculation, the balance values obtained for flow rate are all closed to 1, which are not required to correct. However, the balance values of SS loading deviated from 1±0.1. Hence, the SS concentrations of the discharged primary sludge and excess sludge were corrected by Equation 5-20 and 5-21, respectively. The correction factor is defined as the ratio of [SS concentration after correction]/[SS concentration before correction], and the summary of correction factor is shown in Table 5-8. Along with this, the antibiotics concentration in the solid phase would be corrected by multiplying the measured value by the correction factor.

$$\text{SS concentration of primary sludge after correction (kg/m}^3\text{)} = \frac{SS\ loading_{prim\_inf} - SS\ loading_{prim\_eff}}{Q_{prim\_sludge}}$$

(Equation 5-20)

$$\text{SS concentration of excess sludge after correction (kg/m}^3\text{)} = \frac{SS\ loading_{sec\_inf} - SS\ loading_{sec\_eff}}{(Q_{re\_sludge} + Q_{exc})}$$

(Equation 5-21)

Whereas, $SS\ loading_{prim\_inf} = Q_{prim\_inf} \times SS_{prim\_inf}$; $SS\ loading_{prim\_eff} = Q_{prim\_eff} \times SS_{prim\_eff}$; $SS\ loading_{sec\_inf} = Q_{sec\_inf} \times SS_{sec\_inf}$; $SS\ loading_{sec\_eff} = Q_{sec\_eff} \times SS_{sec\_eff}$.

Table 5-8 The balance of flow rate (Q, m^3/d) and SS loading (kg/d) of two target STPs

STP	Target	Q	SS loading	
			Before correction	Correction factor
STP A	Primary sedimentation	1.00	0.97	-
	Secondary sedimentation	1.00	1.55	0.65
	Entire system	1.00	-	-
STP B_CAS	Primary sedimentation	1.00	0.80	1.33
	Secondary sedimentation	0.93	1.38	0.73
	Entire system	0.95	-	-
STP B_MBR	Entire system	1.00	-	-

5.3.2.3. Removal pathway of measured antibiotics

As calculated by the equations in 5.2.3, the contribution of removal pathways on antibiotics in the two STPs are shown in Figure 5-2. Amoxicillin, ampicillin and cefazolin can be highly removed (>95%) in the three activated sludge systems. Although piperacillin also belongs to β-lactam antibiotics, its removal efficiency was slightly lower than the other three compounds, and the removal of piperacillin in CAS system (88.9%) was lower than those in AAO (93.7%) and MBR (95.5%) system. Previous studies seldom detected the β-lactams, especially penicillins, in the effluent of STPs (Bailón-Pérez et al., 2008; Benito-Peña et al., 2006; Brown et al., 2006), which indicates that β-lactams can be easily removed in the STPs. All β-lactams have a β-lactam ring which can be easily cleaved, while the different side chains may result in the variation of properties (Cha et al., 2006). Therefore, we still need to pay attention on this class of antibiotics who were less removable than others, like piperacillin. The total removal of clarithromycin and azithromycin (macrolides) in STP A and STP B_MBR

were relatively high with a removal efficiency above 85%, however, the removal efficiency of these two compounds were 46.4% and 36.7% in STP B_CAS, respectively. Yasojima et al. (2006) reported a similar removal efficiency of clarithromycin and azithromycin in CAS system, with a value of 43% and 49%, respectively. Kazama (2017) also found that the removal efficiency of clarithromycin and azithromycin in MBR system (clarithromycin: 90%, azithromycin: 97%) were higher than those in CAS system (clarithromycin: 38%, azithromycin: 49%). Narumiya (2011) reported that around 80% of clarithromycin can be eliminated AAO system. For levofloxacin, the removal efficiency in STP A_AAO system (89.6%) are much higher than those in STP B_CAS (64.0%) and STP B_MBR (58.9%) system. Narumiya (2011) also reported high removal of levofloxacin (75-100%) in AAO system. However, Park (2016) reported similar removal efficiency of levofloxacin in AAO (85.6%) and CAS (68.6%) system, while the removal efficiency in MBR system was 83.5%. Kazama (2017) also observed a high removal (around 90%) of levofloxacin in MBR system. The total removal efficiency of sulfamethoxazole and trimethoprim shows similar trends in the three activated sludge system, for sulfamethoxazole: MBR (67.0%) > AAO (51.5%) > CAS (22.3%), for trimethoprim: MBR (84.5%) > AAO (57.6%) > CAS (30.6%). Similar results for these two compounds have been reported in previous studies (Brown et al., 2006; Göbel et al., 2005; Kazama, 2017; Li et al., 2016; Yuan et al., 2019). However, Park (2016) reported a relative low removal (24.3%) of trimethoprim in MBR system.

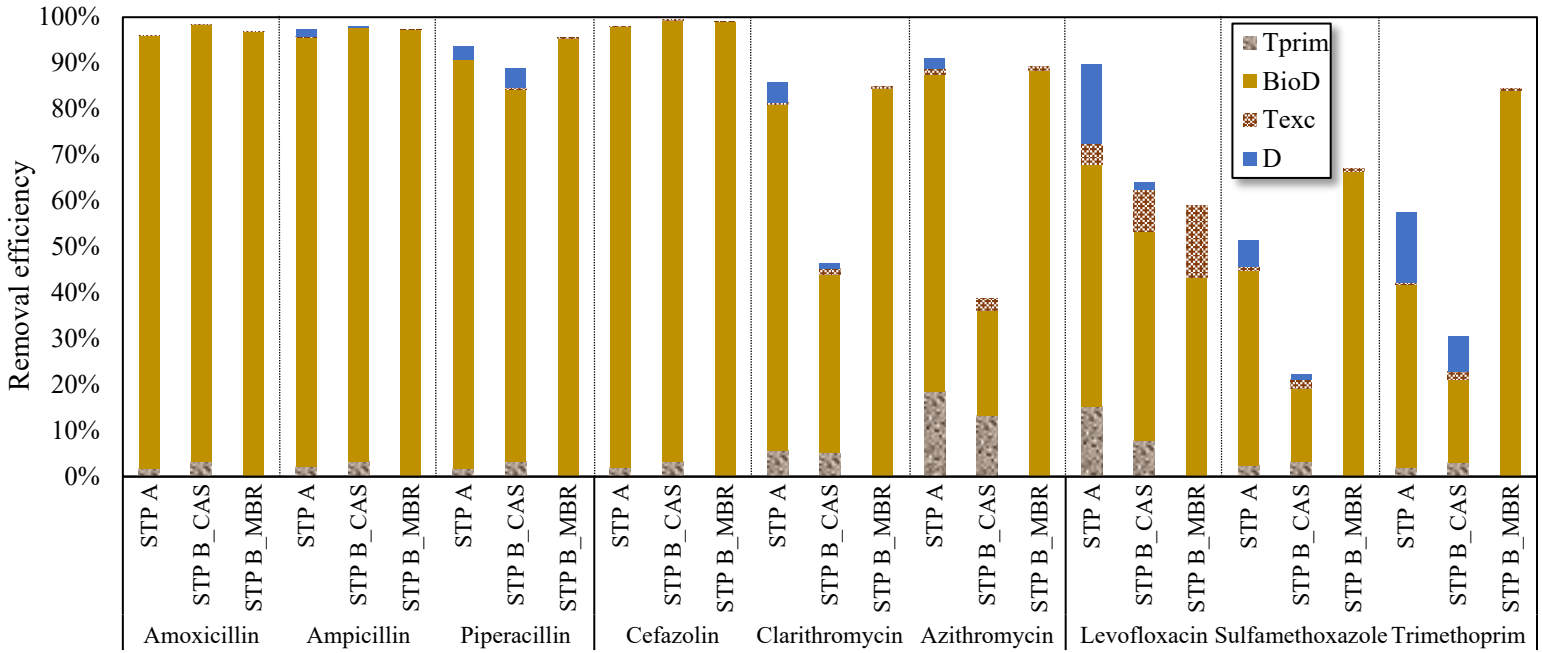


Figure 5-2 The removal pathways of antibiotics in AAO, CAS and MBR systems of two target STPs. Tprim: removal by wasting the primary sludge; BioD: removal by biodegradation; Texc: removal by wasting the excess sludge; D: removal by final treatment.

Removal in primary sedimentation. The removal of antibiotics in the primary treatment is mainly attributed to the adsorption to the primary sludge, hence the removal efficiency is directly related to the sorption ability of target compounds. The removal of target antibiotics by the discharge of primary sludge (T_{prim}) showed similar trends in the two STPs. For the lowly sorptive compounds (amoxicillin, ampicillin, piperacillin, cefazolin, sulfamethoxazole and trimethoprim), the removal efficiencies by the absorption to the primary sludge were below 5%, and they were comparable in the two STPs. Previous researchers also observed no significant elimination for these compounds in the primary sedimentation (Göbel et al., 2007; Gulkowska et al., 2008; Radjenović et al., 2009). For the moderately and highly sorptive compounds (clarithromycin, azithromycin and levofloxacin), the removal efficiency in the primary sedimentation were slightly higher. The T_{prim} of azithromycin is highest among these compounds, with a value of 18.5% and 13.3% in STP A and STP B, respectively, followed by levofloxacin (STP A:15.3%, STP B:7.9%) and clarithromycin (STP A:5.7%, STP B:5.1%). As the results shown, the removal in STP A was higher than that in STP B for all target compounds, which was attributed to the higher sorption distribution coefficient in the primary sludge of STP A. In some STPs, some chemicals were added as the coagulant in the primary treatment, which could achieve higher removal efficiency of some antibiotics at a range of 45-75% (Xu et al., 2007).

Removal in secondary treatment. In the secondary treatment, the target antibiotics were removed by biodegradation (*BioD*) and adsorbed and discharged by excess sludge (T_{exc}). As the results shown, although the properties of antibiotics varied, biodegradation was the main removal pathway for all target compounds. T_{exc} of β-lactam antibiotics was below 0.5%. The removal of amoxicillin, ampicillin and cefazolin was all above 93% in all systems, while their removal in MBR system was slightly higher. However, the removal of piperacillin by biodegradation was slightly lower than other three β-lactams, with a *BioD* value of 89.0%, 81.0% and 95.4% in STP A_AAO, STP B_CAS and STP B_MBR system, respectively. During the batch experiments in 4.3.3, the half-lives of amoxicillin, ampicillin and cefazolin were all <1.5 h in the three activated sludge system, while the half-lives of piperacillin were 1.9-3.8 h (Table 4-3). Cha et al. (2006) proposed that the differences of biodegradability among these β-lactam antibiotics might be result from the diverse side chains on β-lactam ring. For clarithromycin and

azithromycin (macrolides), the removal by biodegradation and adsorption varied by the different activated sludge systems. *BioD* value of these two compounds were highest in MBR system (clarithromycin: 84.5%, azithromycin: 88.5%), followed by those in AAO (clarithromycin: 75.4%, azithromycin: 68.9%) and CAS (clarithromycin: 38.9%, azithromycin: 22.9%) system. Oppositely, T_{exc} of these two compounds were highest in CAS system (clarithromycin: 1.3%, azithromycin: 2.4%), followed by those in AAO (clarithromycin: 0.4%, azithromycin: 1.3%) and MBR (clarithromycin: 0.3%, azithromycin: 0.7%) system. As the results in Table 4-3, the half-lives of clarithromycin and azithromycin in MBR sludge were short (4.8 h, 5.8 h) than those in AAO sludge (7.3 h, 11.3 h) and CAS sludge (16.8 h, 53.3 h), which were coincident to related *BioD* values in the three system. Kazama (2017) obtained similar results of these two compounds in MBR system with a *BioD* value of 88% for clarithromycin and 95% for azithromycin, and with a T_{exc} value of around 2% for both clarithromycin and azithromycin. However, some previous studies observed no significantly removal of macrolides (including clarithromycin and azithromycin) during biological treatment due to their low biodegradability and sorption ability (Göbel et al., 2007; Radjenović et al., 2009). For levofloxacin, the *BioD* value in AAO system (52.8%) was slightly higher than those in CAS (45.5%) and MBR (43.3%) system, while the T_{exc} value in MBR system was highest (15.7%). During batch experiments, there was no significantly biodegradation observed for levofloxacin in the activated sludge from the three systems, and similar results were also reported by Narumiya (2011). The possible reason for the detected biodegradation in this study might be that the biodegradation happened in the solid phase due to large amount and long-term adsorption on to the activated sludge. The *BioD* value and T_{exc} value of sulfamethoxazole and trimethoprim in the three systems showed similar trend to clarithromycin and azithromycin. *BioD* value of these two compounds were highest in MBR system (sulfamethoxazole: 66.3%, trimethoprim: 84.0%), followed by those in AAO (sulfamethoxazole: 42.5%, trimethoprim: 39.8%) and CAS (sulfamethoxazole: 16.0%, trimethoprim: 18.0%) system. While the T_{exc} value of these two compounds in the three system were all below 2%. Previous studies also found the poor removal of sulfamethoxazole and trimethoprim by CAS sludge (Brown et al., 2006; Kazama, 2017; Pérez et al., 2005), and higher removal in AAO and MBR sludge (Kazama, 2017; Park, 2016).

Removal in tertiary treatment. There are two different tertiary treatment units applied in the two STPs. In the STP A, the high-speed sand filter was applied after secondary effluent, while the chlorination disinfection was applied in STP B. In the secondary effluent, there was almost no amoxicillin, ampicillin and cefazolin detected in the two STPs, therefore, the contribution of removal by tertiary treatment (*D*) was neglectable. For piperacillin, the *D* value of chlorination disinfection (4.3%) was higher than that of sand filtration (2.9%). Oppositely, for the other compounds, the *D* values of sand filtration were higher, whereas, the contribution of removal by sand filtration for levofloxacin and trimethoprim reached 17.2% and 15.2%, respectively, followed by sulfamethoxazole (5.7%), clarithromycin (4.5%) and azithromycin (2.2%). Relatively high removal of trimethoprim (60-75%) by sand filtration has been observed by other researchers (Göbel et al., 2005; Göbel et al., 2007; Nakada et al., 2007), and there was no elimination for clarithromycin and azithromycin detected by sand filtration (Nakada et al., 2007). The removal of clarithromycin, azithromycin, levofloxacin and sulfamethoxazole by chlorination were neglectable (<2%) in this study, while it was 7.7% for trimethoprim. Batt et al. (2007) reported that the reduction sulfamethoxazole in concentration ranged from 10 to 70 ng/L after chlorination disinfection, while there was no elimination for trimethoprim. However, higher removal of sulfamethoxazole (81%) and trimethoprim (93%) were observed by Gao et al. (2014) and Lin and Thai (2009). The reason for the serious variations on the removal by chlorination disinfection might be the residual free chlorine concentration during the disinfection units. Li and Zhang (2013) found that there was no further significant removal observed for antibiotics after the residual free chlorine concentration decreased to less than 0.75 mg/L.

5.3.3 Estimation on the fate of antibiotics in target STPs

Based on the models in 5.2.4, the predicted concentrations of target antibiotics in the stream of STP A and STP B are shown in Table 5-9 and 5-10, respectively. The estimated loading fluxes of target antibiotics in the two STPs are given in Figure 5-3 and 5-4, separately. Moreover, the estimated removal efficiencies of antibiotics by primary+secondary treatment in the three activated sludge systems are shown in Figure 5-5. Generally, since the removal of antibiotics by discharge of primary and excess

sludge was limited (Figure 5-3 and 5-4), the estimated removal efficiency of antibiotics principally depended on the biodegradable level of target compounds, which was coincidence to previous studies (Joss et al., 2006; Baalbaki et al., 2016; Taboada-Santos et al., 2020).

Group 1: very highly biodegradable (k_{bio}>5) and lowly sorptive antibiotics (log K_d < 2). This group includes amoxicillin, ampicillin and cefazolin in all activated sludge systems and piperacillin in MBR system, which show a high estimated removal efficiency (Figure 5-5), 89-94% in AAO system, 89-93% in CAS system and 87-95% in MBR system, and the loading fluxes in the biological treatment effluent are very comparable (Figures 5-3, 5-4), which is attributed to the highly biodegradation efficiency in the three activated sludge systems.

Group 2: highly biodegradable (1<k_{bio}<5), lowly and moderately sorptive antibiotics (log K_d < 3). This group includes piperacillin in AAO and CAS system, clarithromycin and azithromycin in MBR system, which show a medium-high removal (60-85%) in the related systems. Their removal was also attributed to the biodegradation (65-75%), and the removal by wasting sludge only accounted for less than 5% (Figures 5-3, 5-4).

Group 3: moderately biodegradable (0.5<k_{bio}<1), lowly and moderately sorptive antibiotics (log K_d < 3). This group includes clarithromycin in AAO and CAS system, azithromycin in AAO system, and trimethoprim in AAO system. They show a medium removal (40-60%) in the related systems, whereas the removal by wasting sludge for moderately sorptive antibiotics (*2 <log K_d < 3*, clarithromycin and azithromycin) accounted for 10-15%, while it was only 2% for lowly sorptive antibiotic (trimethoprim, *log K_d < 2*).

Group 4: hardly biodegradable (k_{bio}<0.5). This group includes levofloxacin and sulfamethoxazole in all activated sludge systems, azithromycin in CAS system, and trimethoprim in CAS and MBR system, which show a low removal (<30%) in related systems regardless their adsorption ability. Since levofloxacin is highly sorptive (*log K_d > 3*) in the sludge, the removal by wasting sludge contributed most for its removal, accounting for 10-15%. However, biodegradation contributed mostly for sulfamethoxazole due to its low sorption ability in the sludge.

Table 5-9 Predicted concentrations (ng/L) of target antibiotics in the stream (influent, primary effluent, primary sludge, excess sludge, and secondary effluent) of STP A

	C_{t_inf} (ng/L)	$C_{t\_prim\_eff}$ (ng/L)	$C_{t\_prim\_sludge}$ (ng/L)	C_{t_exc} (ng/L)	$C_{t\_sec\_eff}$ (ng/L)
Amoxicillin	90	90	121	9	8
Ampicillin	60	60	101	6	5
Piperacillin	431	430	496	110	104
Cefazolin	76	76	79	4	4
Clarithromycin	580	528	5,397	1,144	257
Azithromycin	176	163	1,416	388	101
Levofloxacin	570	540	3,301	3,362	447
Sulfamethoxazole	386	385	523	353	313
Trimethoprim	146	145	199	86	76

Table 5-10 Predicted concentrations (ng/L) of target antibiotics in the stream (influent, primary effluent, primary sludge, excess sludge, and secondary effluent for CAS; influent, excess sludge, and secondary effluent for MBR) of STP B

	CAS					MBR		
	C_{t_inf} (ng/L)	$C_{t\_prim\_eff}$ (ng/L)	$C_{t\_prim\_sludge}$ (ng/L)	C_{t_exc} (ng/L)	$C_{t\_sec\_eff}$ (ng/L)	C_{t_inf} (ng/L)	C_{t_exc} (ng/L)	C_{t_eff} (ng/L)
Amoxicillin	317	316	354	74	25	242	30	12
Ampicillin	235	233	291	47	14	163	26	9
Piperacillin	507	506	533	303	143	440	108	55
Cefazolin	298	298	302	37	12	252	27	14
Clarithromycin	581	529	2,281	1,418	381	491	1,575	127
Azithromycin	237	219	822	573	203	305	927	91
Levofloxacin	1,137	1,079	3,031	5,107	1,111	958	12,005	853
Sulfamethoxazole	233	232	261	249	197	252	280	201
Trimethoprim	126	126	142	147	137	107	132	102

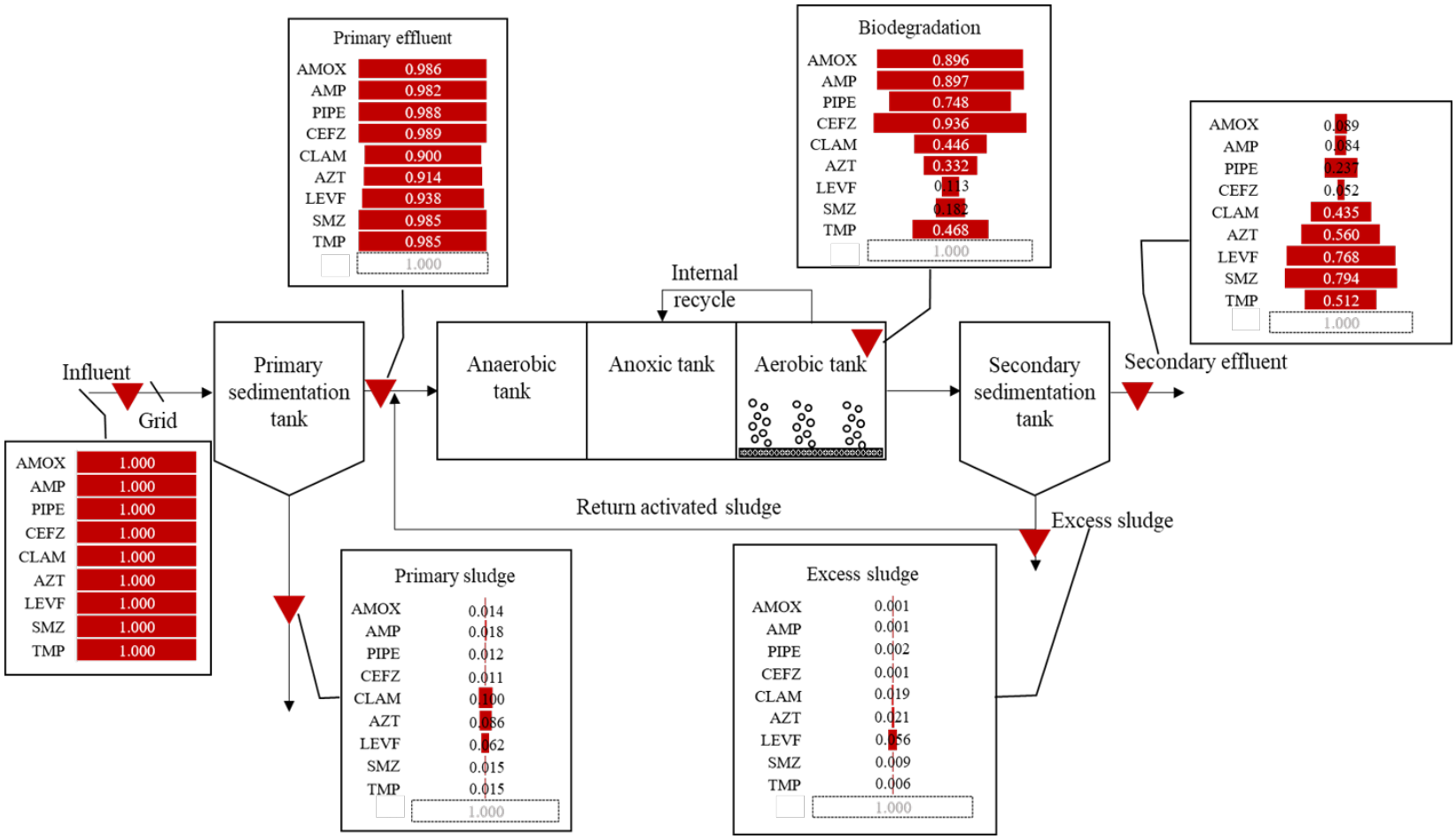


Figure 5-3 The estimated loading fluxes of antibiotics in the stream of STP A, wheraes, the loading of target antibiotics in the influent was considered as 1. AMOX: amoxicillin; AMP: ampicillin; PIPE: piperacillin; CEFZ: cefazolin; AZT: azithromycin; LEVF: levofloxacin; SMZ: sulfamethoxazole; TMP: trimethoprim.

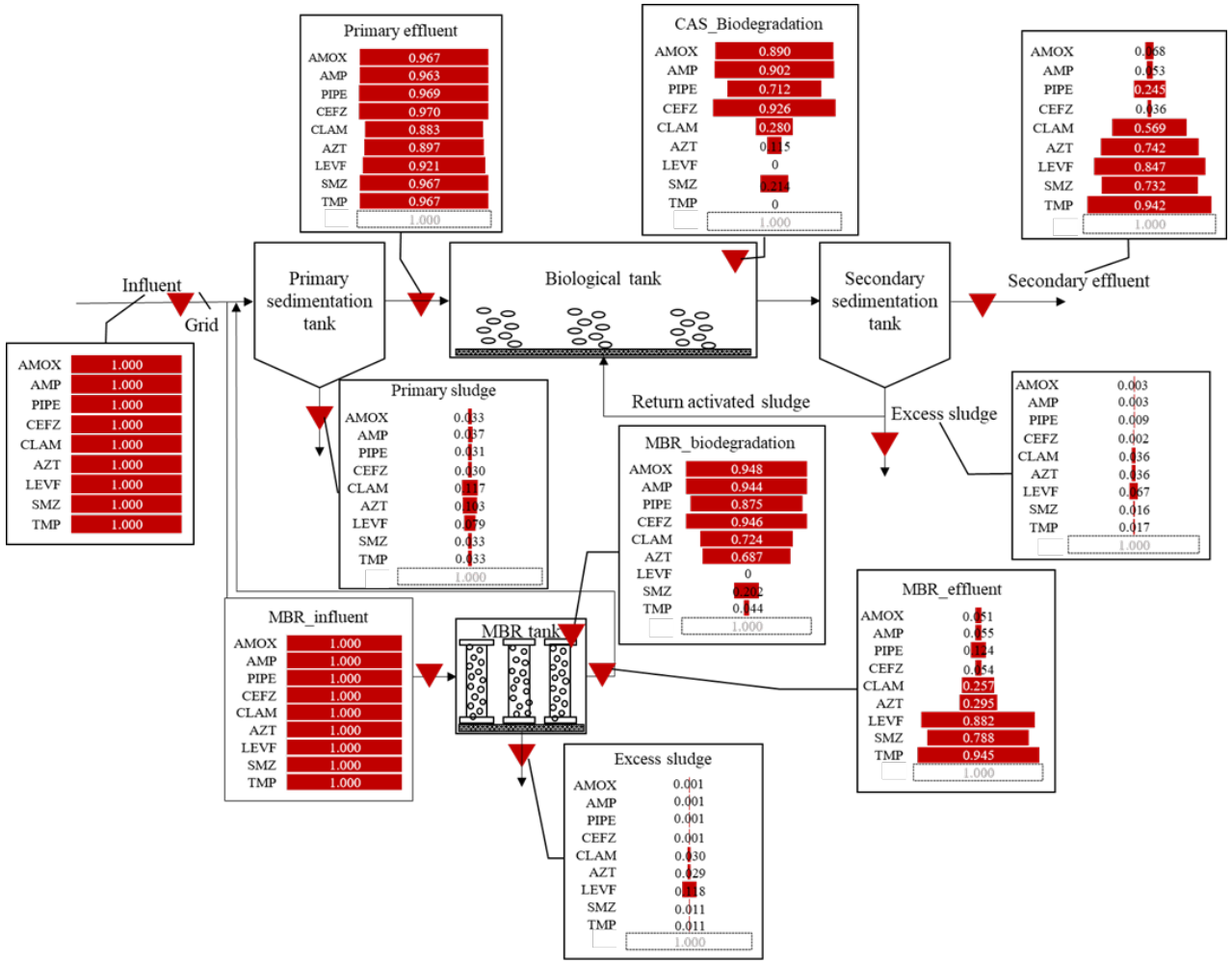


Figure 5-4 The estimated loading fluxes of antibiotics in the stream of STP B, wheraes, the loading of target antibiotics in the influent was considered as 1. AMOX: amoxicillin; AMP: ampicillin; PIPE: piperacillin; CEFZ: cefazolin; AZT: azithromycin; LEVF: levofloxacin; SMZ: sulfamethoxazole; TMP: trimethoprim.

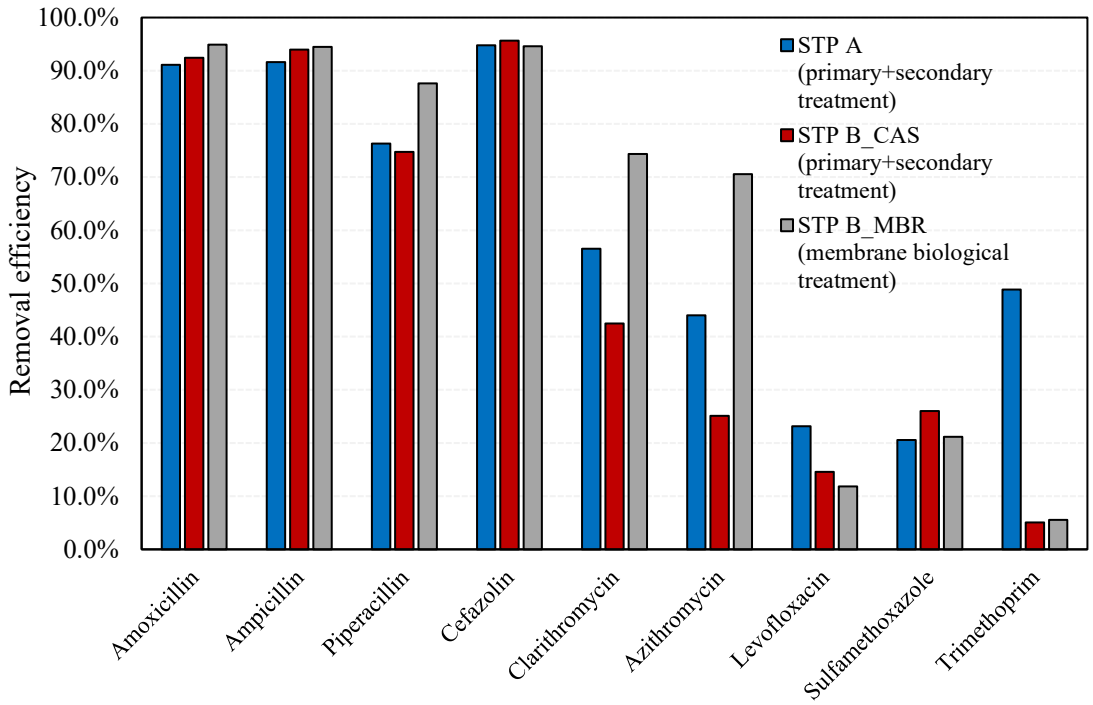


Figure 5-5 Predicted removal efficiency of target antibiotics in the three activated sludge systems of two target STPs

5.3.4 Evaluation on predicted results

Comparison on the predicted and measured removal by wasting primary sludge and excess sludge and biodegradation in the three systems of target STPs are shown in Figure 5-6.

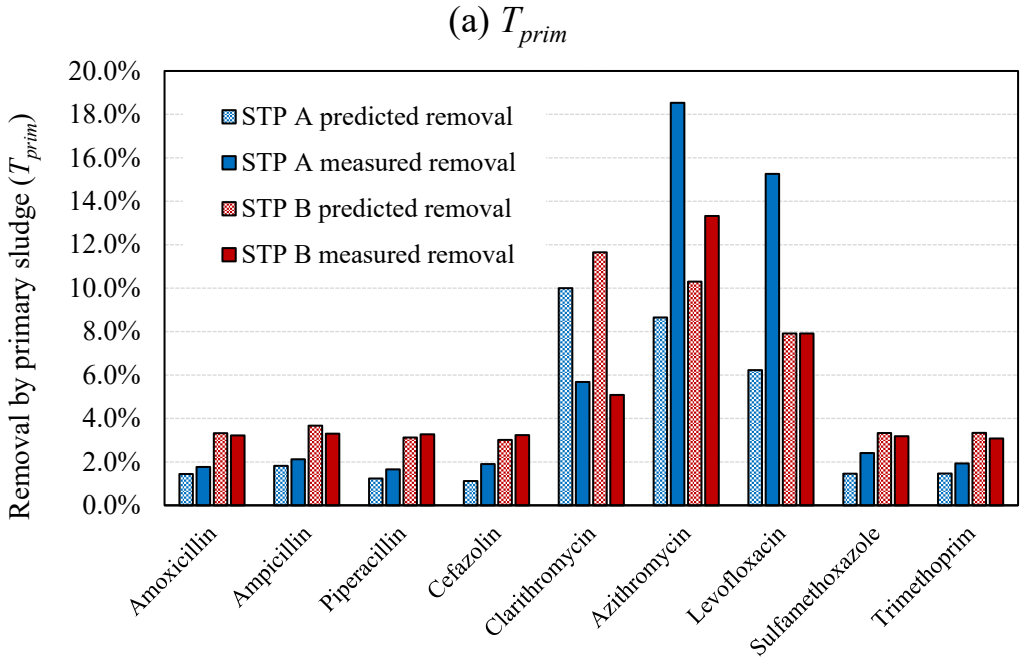
The removal of antibiotics by wasting primary and excess sludge depends on the sorption distribution coefficient (K_d) of target antibiotics, hence, the differences between predicted and measured data resulted from the variations of K_d values applied in the models and measured values. For the lowly sorptive ($log\ K_d < 2$) antibiotics (amoxicillin, ampicillin, piperacillin, cefazolin, sulfamethoxazole and trimethoprim), the differences between predicted and measured total removal by wasting primary and excess sludge varied from -1.0% to 0.7%, which were neglectable compared to the total removal efficiency by the entire system. For the moderately and highly sorptive ($log\ K_d > 2$) antibiotics (clarithromycin, azithromycin and levofloxacin), the differences between predicted and measured removal by wasting excess sludge ranged from -3.8% to 1.6%, which were also acceptable since the ratios of the differences value/total measure

removal efficiency were -6.5-3.4%. However, for the removal by primary sludge, the differences between predicted and measured values range from -9.1% to 8.1%, and the ratios of the differences value/total measure removal efficiency were -10.0-17.5%. The possible reasons might be that 1) the empirical constants of the predictive model for the K_d estimation in 4.3.2 were based on the data in the activated sludge, there might be variations on the sorption performances of antibiotics in primary sludge; 2) the SS concentration of primary sludge always fluctuated seriously and the pretreatment procedures during quantification of antibiotics in solid phase were more complicated than those in liquid phase, therefore, the measured error of antibiotics in solid phase are usually larger than in liquid phase, which could also result in the gap between predicted and measured values of removal by primary sludge. To validate the first assumption, the measured K_d values of target compounds in the influent were applied to calculate the root mean square error (RMSE) between the K_d values applied in the estimation and measured data in primary sludge since the primary sludge comes from the suspended solid of influent. The root mean square error (RMSE) was 0.23 between predicted and measured values, which shows good predictions on the target compounds. It indicated that the empirical predictive models on K_d estimation obtained in 4.3.2 was also proper to apply in primary sludge. It implied that the gap between predicted and measured removal by primary sludge was mostly resulted from the measure error for the adsorption of antibiotics in primary sludge.

The estimated removal by biodegradation in each system was based on the estimated biodegradation rate constant obtained in 4.3.3. Generally, the predicted removal by biodegradation of target antibiotics was less than the measured value. The estimated removal by biodegradation for the very highly biodegradable (k_{bio}>5, group 1 in 5.3.3) antibiotics was acceptable, and the differences between predicted and measured removal by biodegradation ranged from -8.3% to -2.1%. However, the differences between predicted and measured removal by biodegradation varied from -19.8% to -9.8% for the antibiotics in group 2 in 5.3.3 (1<k_{bio}<5), from -35.6% to -7.0% for the antibiotics in group 3 (0.5<k_{bio}<1), and from -79.6% to -6.4% for the antibiotics in group 4 (k_{bio}<1). It indicates that the accuracy of estimation decreased by the decreasing biodegradability of target antibiotics in the activated sludge. In the batch

experiments in Chapter IV, the estimation on the biodegradation rate constants depends on the measured decreasing concentrations of antibiotics in the activated sludge, therefore, the estimation error became larger by the decreasing biodegradability of target compounds, especially for the hardly biodegradable compound, e.g., levofloxacin, sulfamethoxazole, and trimethoprim in CAS and MBR system. To improve the predictive accuracy on the removal of antibiotics by biodegradation, it is essential to improve the estimated accuracy of biodegradation rate constants. One possible way is to extend the reaction time of batch experiment to monitor the decreasing of antibiotics by spiking nutrients for activated sludge in steps.

In summary, the application of estimation models to evaluate the discharging load of antibiotics by secondary effluent of STPs was feasible, however, further improvement is required on the input data, e.g., sorption distribution coefficient (K_d) and biodegradation rate constant (k_{bio}) of target compounds.



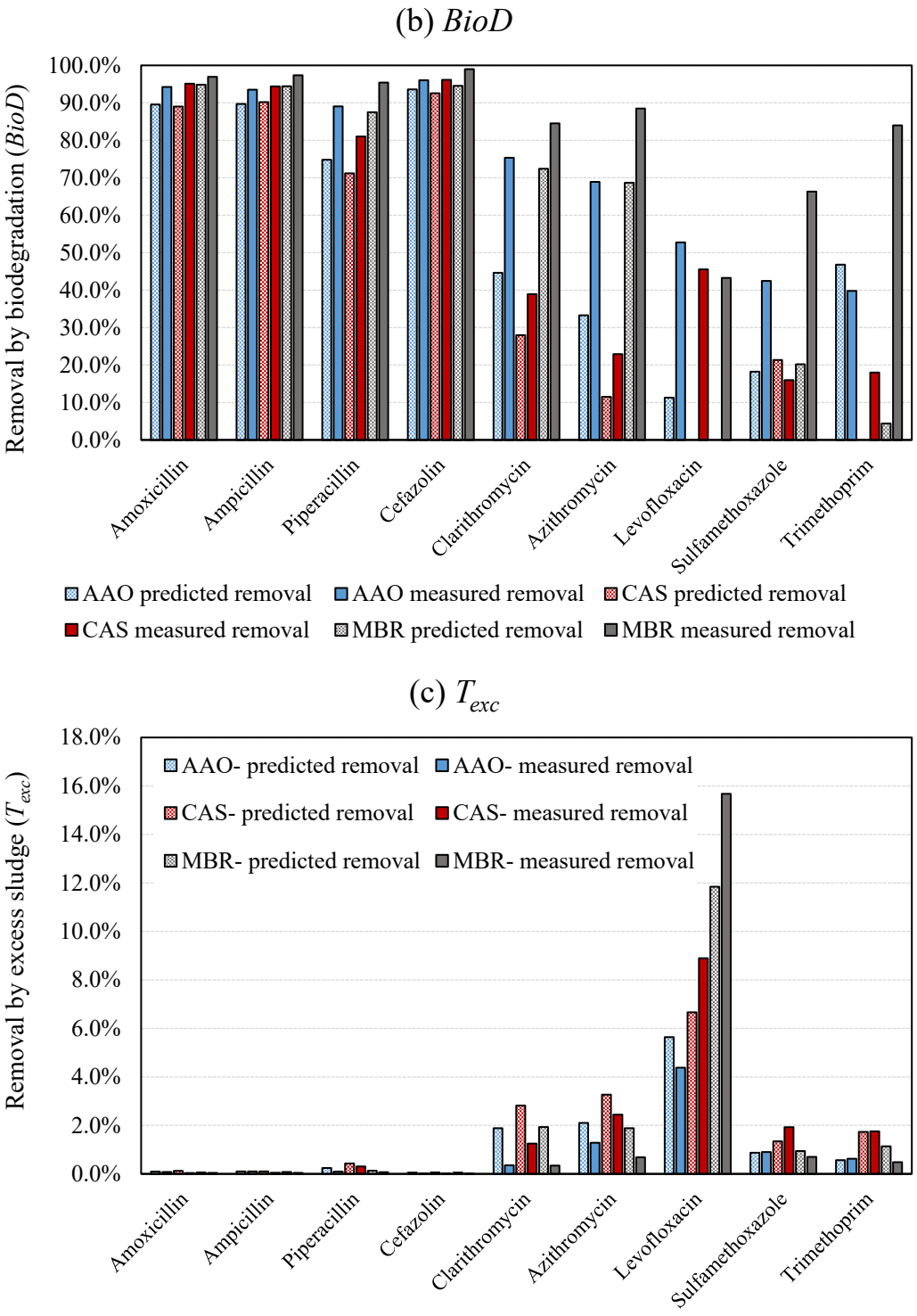


Figure 5-6 Comparison on the predicted and measure removal by (a) primary sludge (T_{prim}), (b) biodegradation (*BioD*) and (c) excess sludge (T_{exc}).

As the results shown in 5.3.3, there could be a classification of the removal based on the classification of sorption distribution coefficient (K_d) and biodegradation rate

constant (k_{bio}) of target compounds. Hence, fifteen virtual compounds (Table 5-11) were set based on K_d and k_{bio} value to estimate the removal in the three systems to make the classification clearer for the removal of antibiotics in the target STPs, whereas, the input influent concentrations of these eight compounds were set to be 1 mg/m^3. On the basis of the estimated results, the classification of estimated removals of target compounds in the three activated sludge system of target STPs are shown in Figure 5-7. For each group, a range of removal efficiency could be obtained, which are summarized in Table 5-12. According this, the following strategy can be obtained: To assess the discharging load of antibiotics for further risk assessment, the classification of antibiotics could be obtained based on estimated/measured K_d value and measured or speculated values of k_{bio} based on the available data of antibiotics similar in structure or from the same class, hence, the range of related estimated removal would be applied to estimate the discharging load of antibiotics.

Table 5-11 Sorption distribution coefficients (K_d), biodegradation rate constant (k_{bio}) and input concentration of fifteen virtual compounds for the study of removal classification

Compound	K_d	k_{bio}, m^3/(kgVSS·d)	Influent concentration (mg/m^3)
Com.1	0	0	1
Com.2	0	0.5	1
Com.3	0	1	1
Com.4	0	5	1
Com.5	0	10	1
Com.6	100	0	1
Com.7	100	0.5	1
Com.8	100	1	1
Com.9	100	5	1
Com.10	100	10	1
Com.11	1000	0	1
Com.12	1000	0.5	1
Com.13	1000	1	1
Com.14	1000	5	1
Com.15	1000	10	1

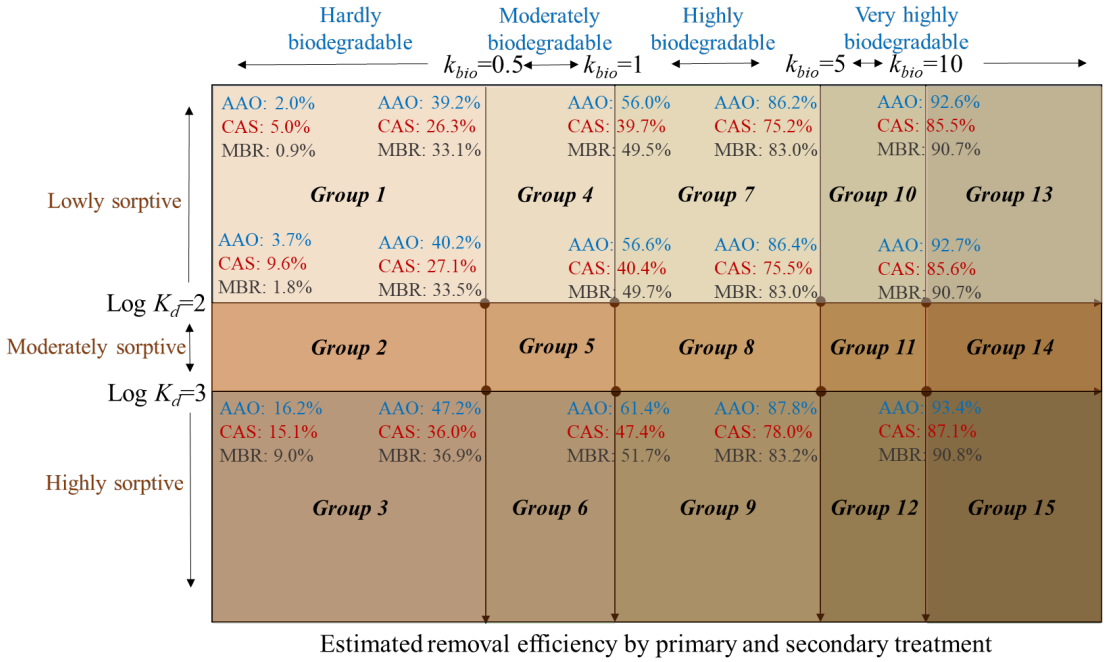


Figure 5-7 Classification of the estimated removal efficiency on the basis of biodegradation and adsorption ability of target compounds.

Table 5-12 Classification of the estimated removal efficiency of antibiotics in target STPs on the basis of biodegradation rate constant (k_{bio}) and sorption distribution coefficients (K_d)

Group	Log K_d	k_{bio}, $m^3/(kgVSS \cdot d)$	Estimated removal efficiency (%)		
			STPA_AAO	STP B_CAS	STP B_MBR
Group 1	<2	<0.5	2.0 - 40.2	5.0 - 27.1	0.9 - 33.5
Group 2	2~3	<0.5	3.7 - 47.2	9.6 - 36.0	1.8 - 36.9
Group 3	>3	<0.5	16.2 - 47.2	15.1 - 36.0	9.0 - 36.9
Group 4	<2	0.5~1	39.2 - 56.6	26.3 - 40.4	33.1 - 49.7
Group 5	2~3	0.5~1	40.2 - 61.4	27.1 - 47.4	33.5 - 51.7
Group 6	>3	0.5~1	47.2 - 51.7	36.0 - 47.4	36.9 - 51.7
Group 7	<2	1~5	56.0 - 86.4	39.7 - 75.7	49.5 - 83.0
Group 8	2~3	1~5	56.6 - 87.8	40.4 - 78.0	49.7 - 83.2
Group 9	>3	1~5	61.4 - 83.2	47.4 - 78.0	51.7 - 83.2
Group 10	<2	5~10	86.2 - 92.7	75.2 - 85.6	83.0 - 90.7
Group 11	2~3	5~10	86.4 - 93.4	75.5 - 87.1	83.0 - 90.8
Group 12	>3	5~10	87.8 - 93.4	78.0 - 87.1	83.2 - 90.8
Group 13	<2	>10	> 92.6	> 85.5	> 90.7
Group 14	2~3	>10	> 92.7	> 85.6	> 90.7
Group 15	>3	>10	> 93.4	> 87.1	> 90.8

5.3.5 PEC_{sec_eff} of antibiotics from target STPs

Since we could not establish proper model to estimate the removal of antibiotics by tertiary treatment, based on the strategy obtained in 5.3.4, the PEC_{inf} of antibiotics in Table 3-6 could be applied to assess the worst-scenario of predicted environmental concentration in secondary effluent (PEC_{sec_eff}) of antibiotics. The classification of target antibiotics and their PEC_{sec_eff} in the two target STPs are summarized in Table 5-13.

In general, the PEC_{sec_eff} of antibiotics in STP A were lower than that in STP B due to the higher wastewater production per inhabitant per day and higher removal efficiency in STP A. The discharge of β-lactams was relatively low even the total consumption was high in Japan, which was resulted from the highly degradable character. It has been reported that β-lactam antibiotics were mainly transformed via the hydrolysis of β-lactam ring and the transformation products could be the final products or be further degraded (Mayers, 2009). Therefore, for the further risk assessment, it is necessary to consider the toxicity and antimicrobial activity of the transformation products of β-lactams. For the antibiotics from other classes, even the removal in AAO system were higher than that in CAS system, the PEC_{sec_eff} from the target STP A were still high, which require further advanced treatment showed higher removal of antibiotics, e.g., ozonation (Nakada et al., 2007). As the results in this study and some previous study, MBR can improve the biodegradation ability or shorten the half-lives of antibiotics due to the higher microbial diversity and MLSS, which could be considered as an alternative for the further STP upgrading.

Table 5-13 Classification of antibiotics and predicted environmental concentration of secondary effluent in the two target STPs

	PEC_{inf} (ng/L)		Classification		Estimated removal efficiency (%)		PEC_{sec_eff} (ng/L)	
	STP A	STP B	STP A	STP B	STP A	STP B	STP A	STP B
Amoxicillin	855-1200	1115-1561	***Group 10***	***Group 13***	86.2 - 92.7	> 85.5	64-170	0-261
Ampicillin	184-245	203-271	***Group 10***	***Group 13***	86.2 - 92.7	> 85.5	14-35	0-45
Piperacillin	446-545	577-705	***Group 7***	***Group 7***	56.0 - 86.4	39.7 - 75.7	62-250	162-490
Cefazolin	337-404	529-635	***Group 13***	***Group 13***	> 92.6	> 85.5	0-31	0-106
Cephalexin	63	79	***Group 10***	***Group 10***	86.2 - 92.7	75.2 - 85.6	5-9	13-23
Cefaclor	102-132	86-111	***Group 10***	***Group 10***	86.2 - 92.7	75.2 - 85.6	8-20	14-32
Cefmetazole	80-97	119-143	***Group 10***	***Group 10***	86.2 - 92.7	75.2 - 85.6	6-10	20-41
Cefotiam	24-33	38-51	***Group 10***	***Group 10***	86.2 - 92.7	75.2 - 85.6	2-5	6-15
Cefcapene	321-398	311-387	***Group 10***	***Group 10***	86.2 - 92.7	75.2 - 85.6	24-56	52-111
Cefditoren	95-105	163-182	***Group 10***	***Group 10***	86.2 - 92.7	75.2 - 85.6	7-15	27-52
Ceftriaxone	131-210	215-345	***Group 10***	***Group 10***	86.2 - 92.7	75.2 - 85.6	10-30	36-99
Cefdinir	24-47	37-74	***Group 10***	***Group 10***	86.2 - 92.7	75.2 - 85.6	2-7	6-20
Meropenem	150-162	187-202	***Group 10***	***Group 10***	86.2 - 92.7	75.2 - 85.6	11-23	31-58
Clarithromycin	512-1140	587-1300	***Group 5***	***Group 5***	40.2 - 61.4	27.1 - 47.4	202-695	356-1,100
Azithromycin	166-554	201-671	***Group 5***	***Group 2***	40.2 - 61.4	9.6 - 36.0	65-340	148-700
Sulfasalazine	267	338	***Group 2***	***Group 2***	3.7 - 47.2	9.6 - 36.0	144-262	249-352
Levofloxacin	716-818	1149-1313	***Group 3***	***Group 3***	16.2 - 47.2	15.1 - 36.0	386-700	848-1,290
Tosufloxacin	47-81	88-152	***Group 3***	***Group 3***	16.2 - 47.2	15.1 - 36.0	25-69	65-150
Sulfamethoxazole	138-184	130-173	***Group 1***	***Group 1***	2.0 - 40.2	5.0 - 27.1	84-180	109-190
Trimethoprim	79-110	74-104	***Group 4***	***Group 1***	39.2 - 56.6	5.0 - 27.1	35-68	62-110

5.4 Conclusions

In this Chapter, the investigation on the fate of target antibiotics were carried out in the two target STPs. The integration of the primary and secondary treatment models was applied to estimate the fate of antibiotics in the STPs. After evaluate the results by measured data, a strategy for the prediction of antibiotics concentration in secondary effluent of target STPs was achieved and applied to obtain the PEC_{sec_eff}. The major findings from this study were as follows:

- ✓ All the target antibiotics can be detected in influent and primary effluent. The concentrations of amoxicillin, ampicillin and cefazolin in secondary of effluent and effluent were below the limits of detection (LOD) in the two STPs. In STP A, the highest average concentration of target antibiotics detected in the effluent was for sulfamethoxazole (263 ng/L), and the concentrations of other compounds detected in the effluent were all lower than 100 ng/L. In STP B, the concentrations of all target compounds in MBR-effluent were lower than those in CAS-effluent.
- ✓ The removal of target antibiotics by the discharge of primary sludge (T_{prim}) showed similar trends in the two STPs. For the lowly sorptive compounds (amoxicillin, ampicillin, piperacillin, cefazolin, sulfamethoxazole and trimethoprim), the removal efficiencies by the absorption to the primary sludge were below 5%, and they were comparable in the two STPs. For the moderately and highly sorptive compounds (clarithromycin, azithromycin and levofloxacin), the removal efficiency in the primary sedimentation were slightly higher.
- ✓ In the secondary treatment, biodegradation was the main removal pathway for all target compounds. The removal of amoxicillin, ampicillin and cefazolin was all above 93% in all systems, while their removal in MBR system was slightly higher. However, the removal of piperacillin by biodegradation was slightly lower than other three β-lactams, with a *BioD* value of 89.0%, 81.0% and 95.4% in STP A_AAO, STP B_CAS and STP B_MBR system, respectively. The biodegradation of clarithromycin and azithromycin were highest in MBR system, followed by those in AAO and CAS system. For levofloxacin, the *BioD* value in AAO system (52.8%) was slightly higher than those in CAS (45.5%) and MBR (43.3%) system;

The *BioD* value and T_{exc} value of sulfamethoxazole and trimethoprim in the three systems showed similar trend to clarithromycin and azithromycin.

- ✓ The removal of piperacillin by chlorination disinfection (4.3%) was higher than that by sand filtration (2.9%). Oppositely, for the other compounds, the removal by sand filtration were higher, whereas, the contribution of removal by sand filtration for levofloxacin and trimethoprim reached 17.2% and 15.2%, respectively, followed by sulfamethoxazole (5.7%), clarithromycin (4.5%) and azithromycin (2.2%).
- ✓ The estimated removal of antibiotics by wasting primary and excess sludge were acceptable, while for the biodegradation, the accuracy of predicted removal for the moderately and hardly biodegradable antibiotics were relatively low, which was mainly resulted from the lower estimated accuracy of biodegradation rate constant (k_{bio}) in Chapter IV.
- ✓ A fifteen-group classification on the estimated removal efficiency based on the sorption distribution coefficient (K_d) and biodegradation rate constant (k_{bio}) of antibiotics was established and applied to obtain the PEC_{sec_eff} of antibiotics in target STPs.

5.5 Reference

Alvarino, T., Suarez, S., Lema, J. M., Omil, F., 2014. Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. *Journal of Hazardous Materials*, *278*, pp.506-513.

Baalbaki, Z., Sultana, T., Maere, T., Vanrolleghem, P. A., Metcalfe, C. D., Yargeau, V., 2016. Fate and mass balance of contaminants of emerging concern during wastewater treatment determined using the fractionated approach. *Science of the Total Environment*, *573*, pp.1147-1158.

Bailón-Pérez, M.I., García-Campaña, A.M., Cruces-Blanco, C., del Olmo Iruela, M., 2008. Trace determination of β-lactam antibiotics in environmental aqueous samples using off-line and on-line preconcentration in capillary electrophoresis. *Journal of Chromatography A*, *1185*(2), pp.273-280.

Batt, A. L., Kim, S., Aga, D. S., 2007. Comparison of the occurrence of antibiotics in four full-scale wastewater treatment plants with varying designs and operations. *Chemosphere*, *68*(3), pp.428-435.

Benito-Peña, E., Partal-Rodera, A.I., León-González, M.E., Moreno-Bondi, M.C., 2006. Evaluation of mixed mode solid phase extraction cartridges for the preconcentration of β-lactam antibiotics in wastewater using liquid chromatography with UV-DAD detection. *Analytica Chimica Acta*, *556*(2), pp.415-422.

Brown, K.D., Kulis, J., Thomson, B., Chapman, T.H., Mawhinney, D.B., 2006. Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. *Science of the Total Environment*, *366*(2-3), pp.772-783.

Carballa, M., Fink, G., Omil, F., Lema, J. M., Ternes, T., 2008. Determination of the solid-water distribution coefficient (K_d) for pharmaceuticals, estrogens and musk fragrances in digested sludge. *Water Research*, *42*(1-2), pp.287-295.

Cha, J.M., Yang, S., Carlson, K.H., 2006. Trace determination of β-lactam antibiotics in surface water and urban wastewater using liquid chromatography combined with electrospray tandem mass spectrometry. *Journal of Chromatography A*, *1115*(1-2), pp.46-57.

Ferreira, V.R., Amorim, C.L., Cravo, S.M., Tiritan, M.E., Castro, P.M., Afonso, C.M., 2016. Fluoroquinolones biosorption onto microbial biomass: activated sludge and aerobic granular sludge. *International Biodeterioration & Biodegradation*, *110*, pp.53-60.

Gao, S., Zhao, Z., Xu, Y., Tian, J., Qi, H., Lin, W., Cui, F., 2014. Oxidation of sulfamethoxazole (SMX) by chlorine, ozone and permanganate—A comparative study. *Journal of Hazardous Materials*, *274*, pp.258-269.

Gernaey, K. V., Jeppsson, U., 2014. *Benchmarking of control strategies for wastewater treatment plants*. IWA publishing.

Gulkowska, A., Leung, H. W., So, M. K., Taniyasu, S., Yamashita, N., Yeung, L. W., Bruce, J. R., Lei, A. P., Giesy, J. P., Lam, P. K., 2008. Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China. *Water Research*, *42*(1-2), pp.395-403.

Guo, L., Vanrolleghem, P. A., 2014. Calibration and validation of an activated sludge model for greenhouse gases no. 1 (ASMG1): prediction of temperature-dependent N 2 O emission dynamics. *Bioprocess and Biosystems Engineering*, *37*(2), pp.151-163.

Göbel, A., McArdell, C. S., Joss, A., Siegrist, H., Giger, W., 2007. Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies. *Science of the Total Environment*, *372*(2-3), pp.361-371.

Göbel, A., Thomsen, A., McArdell, C. S., Joss, A., Giger, W., 2005. Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. *Environmental Science & Technology*, *39*(11), pp.3981-3989.

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. *Water Research*, *40*(8), pp.1686-1696.

Kazama S., 2017. Removal characteristics and mechanisms of pharmaceuticals in membrane bioreactor. (Master's Thesis) Graduate School of Engineering, Kyoto University, Japan.

Le-Minh, N., Khan, S. J., Drewes, J. E., Stuetz, R. M., 2010. Fate of antibiotics during municipal water recycling treatment processes. *Water Research*, *44*(15), pp.4295-4323.

Li, B., Zhang, T., 2013. Different removal behaviors of multiple trace antibiotics in municipal wastewater chlorination. *Water Research*, *47*(9), pp.2970-2982.

Li, J., Cheng, W., Xu, L., Jiao, Y., Baig, S. A., Chen, H., 2016. Occurrence and removal of antibiotics and the corresponding resistance genes in wastewater treatment plants: effluents' influence to downstream water environment. *Environmental Science and Pollution Research*, *23*(7), pp.6826-6835.

Lin, A. Y. C., Tsai, Y. T., 2009. Occurrence of pharmaceuticals in Taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities. *Science of the Total Environment*, *407*(12), pp.3793-3802.

Mayers, D.L. ed., 2009. *Antimicrobial drug resistance* (pp. 803-824). New York: Humana press.

Nakada, N., Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N., Takada, H., 2007. Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant. *Water Research*, *41*(19), pp.4373-4382.

Narumiya, M., 2011. Simultaneous quantification of PPCPs and their fate in municipal wastewater treatment plants. (Master's Thesis) Graduate School of Engineering, Kyoto University, Japan.

Oberoi, A. S., Jia, Y., Zhang, H., Khanal, S. K., Lu, H., 2019. Insights into the fate and removal of antibiotics in engineered biological treatment systems: a critical review. *Environmental science & technology*, *53*(13), pp.7234-7264.

Ort, C., Lawrence, M. G., Rieckermann, J., Joss, A., 2010. Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review. *Environmental Science & Technology*, *44*(16), pp.6024-6035.

Park J., 2016. Removal characteristics and predictive model of pharmaceuticals and personal care products (PPCPs) in membrane bioreactor (MBR) process. Doctoral Thesis) Graduate School of Engineering, Kyoto University, Japan.

Pérez, S., Eichhorn, P., Aga, D. S., 2005. Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. *Environmental Toxicology and Chemistry: An International Journal*, *24*(6), pp.1361-1367.

Radjenović, J., Petrović, M., Barceló, D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. *Water research*, *43*(3), pp.831-841.

Taboada-Santos, A., Behera, C. R., Sin, G., Gernaey, K. V., Mauricio-Iglesias, M., Carballa, M., Lema, J. M., 2020. Assessment of the fate of organic micropollutants in novel wastewater treatment plant configurations through an empirical mechanistic model. *Science of The Total Environment*, *716*, pp.137079.

Taboada-Santos, A., Braz, G. H., Fernandez-Gonzalez, N., Carballa, M., Lema, J. M., 2019. Thermal hydrolysis of sewage sludge partially removes organic micropollutants but does not enhance their anaerobic biotransformation. *Science of The Total Environment*, *690*, pp.534-542.

Vasudevan, D., Bruland, G.L., Torrance, B.S., Upchurch, V.G., MacKay, A.A., 2009. pH-dependent ciprofloxacin sorption to soils: interaction mechanisms and soil factors influencing sorption. *Geoderma*, *151*(3-4), pp.68-76.

Wang, J., Chu, L., Wojnárovits, L., Takács, E., 2020. Occurrence and fate of antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) in municipal wastewater treatment plant: an overview. *Science of The Total Environment*, pp.140997.

Xu, W., Zhang, G., Li, X., Zou, S., Li, P., Hu, Z., Li, J., 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. *Water Research*, *41*(19), pp.4526-4534.

Yasojima, M., Nakada, N., Komori, K., Suzuki, Y., Tanaka, H., 2006. Occurrence of levofloxacin, clarithromycin and azithromycin in wastewater treatment plant in Japan. *Water Science and Technology*, *53*(11), pp.227-233.

Yuan, S. F., Liu, Z. H., Yin, H., Dang, Z., Wu, P. X., Zhu, N. W., Lin, Z., 2019. Trace determination of sulfonamide antibiotics and their acetylated metabolites via SPE-LC-MS/MS in wastewater and insights from their occurrence in a municipal wastewater treatment plant. *Science of the Total Environment*, *653*, pp.815-821.

Zhang, T., Li, B., 2011. Occurrence, transformation, and fate of antibiotics in municipal wastewater treatment plants. *Critical Reviews in Environmental Science and Technology*, *41*(11), pp.951-998.

Chapter VI

Risk assessment and discharge limit proposed for the antibiotics discharged from STPs on the perspective of environmental and human health

6.1 Introduction

Since antibiotics are designed for the treatment of bacterial infection, after excretion from human bodies, there are potential risks on three main parts. Firstly, it might influence the microorganisms in activated sludge of STPs. Secondly, antibiotics cannot be completely removed in STPs and would be released to the aquatic environment, it may impact the non-target organisms which have vital functions of the ecosystem and disrupt the ecosystem. Moreover, the antibiotic residues have potential effects on resistance in the environment. The environmental risk assessment (ERA) proposed by EMEA (2018) aims to establish the safe concentrations for the protection of ecosystem by the calculations of ecotoxicity [e.g., predicted no effect concentration (PNEC)] of micropollutants, including antibiotics. In Europe, the ERA is required if the predicted environmental concentration (PEC) of a medicine exceeds 10 ng/L. The $PNEC_{microorganism}$ is applied to evaluated the risk of antibiotics to activated sludge in STPs based on the "Activated sludge respiration inhibition test" (ASRIT) (OECD, 2010). Due to many factors including short exposure time (3h), the available ASRIT results are reported as censored data of above 100 mg/L (Brandt et al., 2015), which are much higher than the PEC_{inf} value in Chapter 3. Therefore, it is not possible to applied the available ASRIT results to evaluate the potential risk of antibiotics in STPs. For the ecotoxicity assessment, in the current ERA framework, cyanobacteria were assumed to be the most sensitive species for some compounds, such as antibiotics, therefore, the PNEC of surface water ($PNEC_{sw}$) is calculated from the toxicity to cyanobacteria [no observed effect concentrations (NOECs) or 10% effect concentrations (EC10s)] (EMEA, 2018).

It has been reported that microorganisms can develop the antimicrobial resistance (AMR) under a low exposure of antibiotics (World Health Organization, 2014), which is a major threat to public health. For the protection of human health, the minimum inhibitory concentrations (MICs), the lowest concentration for no observed growth, were monitored in clinically relevant bacteria (CRB), which were collected in the European Committee on Antimicrobial Susceptibility Testing database (http://www.eucast.org). In fact, the minimum selective concentrations (MSCs), the lowest concentration that will select for AMR, were up to several hundred-fold below the related MICs (Gullberg et al., 2011; Sandegren, 2019).

Since the current ERA framework conducted in one species of cyanobacteria only, some other species (e.g., green algae, macrophytes, invertebrates and so on) were also demonstrated to be more sensitive to some antibiotics than cyanobacteria (Le Page et al., 2017; Wess et al., 2020). It is essential to collect the reliable NOECs or EC10s of toxicity test of antibiotics on different species and evaluate the sensitivity to obtain the lowest concentrations of $PNEC_{sw}$. Furthermore, the potentially enrichment concentration of AMR may be lower than the concentration of ERA ecotoxicity inhibition tests (Bengtsson-Palme and Larsson, 2016; Le Page et al., 2017). For the perspective of both environmental protection and human health, it is essential to incorporate the traditional ERA ($PNEC_{sw}$) with the AMR selection ($PNEC_R$) to assess the potential risk.

In this Chapter, the aquatic ecotoxicity on different species and MIC of target antibiotics were collected from previous literatures to assess the sensitivity; the lowest NOEC or EC10s were applied to calculate the $PNEC_{sw}$, which were compared to the $PNEC_R$ calculated on the basis of MICs; risk assessment was carried out based on the PNEC results and discharge limits were proposed for the prospective of environmental and human health; finally, the possible strategies for the achievement of discharge target were proposed.

6.2 Materials and Methods

6.2.1 Data collection

For the ecological risk assessment, to evaluate the sensitivity of antibiotics on different species, it is important to collect the reliable results from previous studies of toxicity tests for target antibiotics on different species. For the data before 2017, we directly took from Le Page et al. (2017), which collected the reliable data for antibiotics on the commonly used species in ERA list (including cyanobacteria, green algae, macrophytes, invertebrates and fish); for the data after 2017, we used *Google Scholar* search with "Antibiotic toxicity test" AND "OECD 201" OR "ISO8962" OR "ISO 8962" OR "850.4500" OR "E1440-91" following the same criteria to Le Page et al. (2017). The lowest NOEC or EC10s from literatures were applied to compare the sensibility among different species.

For the MSC, it is notice worthy that until now the experimental studies of MSCs are limited, and there is no standardized test method for MSCs, the reliability of experimental MSCs is still limited. For the urgent requirement of establishment on risk assessment and emission limits based on the AMR issue, Bengtsson-Palme and Larsson (2016) calculated a size-adjusted lowest MIC, which is a theoretical adjustment to the MIC to include 99% of CRB based on the EUCAST database (http://www.eucast.org). However, the EUCAST database kept updating the data and added more tested species to some antibiotics since the latest accessed by Bengtsson-Palme and Larsson (2016) on 2014-11-26. The number of tested species is a factor for calculating the size-adjusted lowest MIC, therefore, in this study, the size-adjusted lowest MICs would be calculated by applying the latest number of tested species (accessed on 2021-09-21) for target antibiotics according the formula obtained by Bengtsson-Palme and Larsson (2016), and the latest size-adjusted lowest MICs were applied to estimate the theoretical MSCs. It has been reported that the MSC varied between 1/4 and 1/230 (with a median of 1/10) of the MIC depending on the antibiotic and the type of resistance mutation examined (Gullberg et al., 2011; Liu et al., 2011; Westhoff et al, 2017), in this study, the MSCs were estimated by applying a factor of 10 (median value) to the size-adjusted lowest MICs.

6.2.2 PNEC calculation

Since the latest revised version of guideline on the ERA reported that the EC10s is preferred over the NOEC for the calculation of PNEC even if the former is higher than the latter (EMEA, 2018). Therefore, $PNEC_{sw}$ was calculated by applying an assessment factor of 10 to the lowest EC10s or NOEC value from the most sensitive species (EMEA, 2018; von der Ohe et al., 2011).

For the calculation of $PNEC_R$, it is important to applied the equivalent endpoint to the calculation of $PNEC_{sw}$, otherwise, it may lead to the misunderstanding of the sensibility between species in traditional ERA and CRB (Bengtsson-Palme and Larsson, 2018). According to the growth curve in Gullberg et al. (2011), MSC depends on the differences between the growth curve of resistant strain and susceptible strain, in this study, similar to the $PNEC_{sw}$ calculation in traditional ERA, $PNEC_R$ was calculated by applying an assessment factor of 10 to the estimated MSC by 6.2.1, which is different from Le Page et al. (2017), which actually directly applied the theoretical MSC in Bengtsson-Palme and Larsson (2016) as $PNEC_R$ value.

6.2.3 Risk quotient (*RQ*) characterization of PEC_{sec_eff}

To characterize the potential risk of the secondary effluent from the two target STPs, the lowest PNEC ($PNEC_L$) and the predicted environmental concentration in secondary effluent (Table 5-13) of target antibiotics were applied to calculate the risk quotient (*RQ*) by following equation (EAMA, 2018):

$$RQ = \frac{PEC_{sec\_eff}}{PNEC_L} \qquad \text{(Equation 6-1)}$$

If RQ > 1, it shows high potential risk and needs further tertiary treatment, or it would be recommended to reduce the discharged concentration through the improvement of removal during secondary treatment or the reduction on consumption.

6.3 Results and Discussions

Since neither ecotoxicity test data nor MIC data of cefmetazole, cefotiam, cefcapene, cefditoren, sulfasalazine and tosufloxacin were found from previous literature or databases, these compounds were not involved in the risk assessment.

6.3.1 Sensibility of target antibiotics among traditional ERA species

In current ERA, cyanobacteria are considered to be more appropriate since it is assumed as the most sensitive type, especially for antibiotics (EAMA, 2018). In this study, the sensibility of previous ecotoxicity test among traditional ERA species were compared by using the investigated lowest NOEC or EC10s in previous literature (Figure 6-1). Besides the antibiotics mentioned above, there was no ecotoxicity test data available for piperacillin, ceflactor and cefdinir. Cyanobacteria is the most sensitive for β-lactams (amoxicillin, ampicillin, cefazolin, ceftriaxone, cephalexin and meropenem) and macrolides (azithromycin and clarithromycin) due to the lower NOEC or EC10s. According the available species information of cyanobacteria, *Anabaena flos-aquae* is the most sensitive for cefazolin, meropenem and clarithromycin, while *Microcystis aeruginosa* is the most sensitive for ampicillin, and *Synechococcus leopoliensis* for amoxicillin, which indicates that the sensibility varied by species even for the same type (cyanobacteria). Le Page et al. (2017) also found that cyanobacteria is the most sensitive for some other macrolide antibiotics (erythromycin, lincomycin and tylosin). However, *Lemna gibba* (macrophytes) is the most sensitive species for levofloxacin and trimethoprim. Except for levofloxacin, cyanobacteria are still reported to be the most sensitive for some other quinolones (ofloxacin, norfloxacin and enrofloxacin) (Le Page et al., 2017), it means that the sensibility varied even for the same class of antibiotics. In this study, cyanobacteria, macrolytes and invertebrates are comparable for sulfamethoxazole, while cyanobacteria are considered to be not appropriate for sulfonamides during risk assessment since it was less sensitive than microalgae or macrophytes (Le Page et al., 2017). Among these traditional ERA types, fish and invertebrates are less sensitive compared to other species by several orders of magnitude which would not be used in the risk assessment for antibiotics.

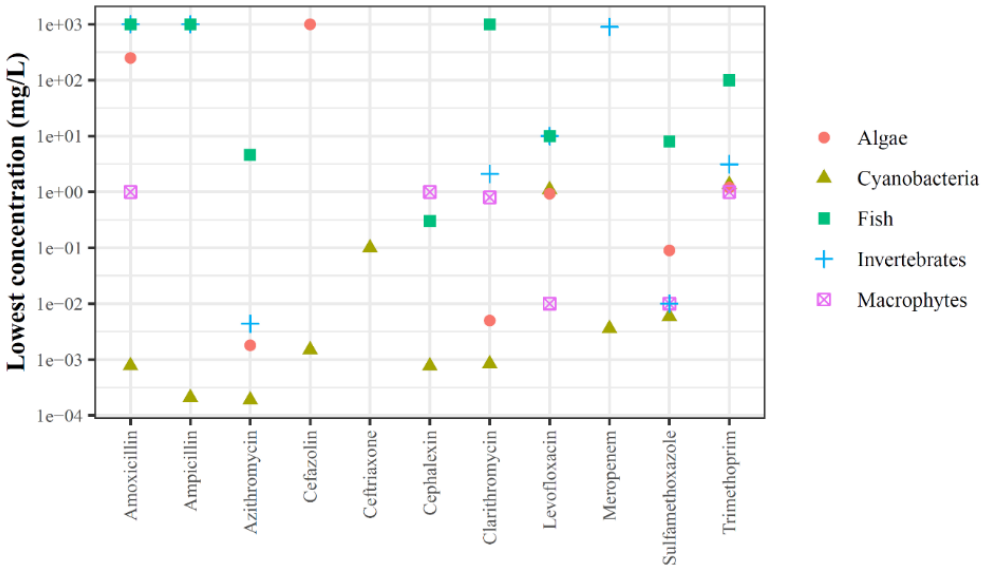


Figure 6-1 Comparison of lowest concentration of NOEC or EC10s of antibiotics on different traditional ERA species.

Overall, cyanobacteria are not the most sensitive species for all antibiotics, the sensibility varied by species and individual compound. Therefore, during the risk assessment work, even for traditional ERA, we should apply the ecotoxicity test data depending on the sensibility for individual antibiotic. However, the ecotoxicity data on some types (e.g., algae, macrophytes) were not available for some antibiotics, e.g., ampicillin, cefazolin, ceftriaxone and so on, and it might lead to the unreliability of the results. Moreover, even for the same type like cyanobacteria, there are insufficient species applied for the ecotoxicity test. For the improvement of the reliability in the risk assessment work and fully protective of the diversity of species in the environment, it is still essential to applied more species in the ecotoxicity test.

6.3.2 Estimated MSCs of target antibiotics

The estimated MSCs of target antibiotics were calculated by the size-adjusted MICs based on the last accessed data in EUCAST according to Bengtsson-Palme and Larson (2016) and shown in Table 6-1. Overall, the estimated MSCs of target antibiotics ranged from 0.2 µg/L (ceftriaxone and meropenem) to 26.8 µg/L (sulfamethoxazole). For the antibiotics (amoxicillin, ampicillin, piperacillin, ceftriaxone, meropenem and levofloxacin) with a number of tested species more than 40, the estimated MSCs were

relatively low with a range of 0.2-0.4 μg/L, while which were higher for the antibiotics with a smaller number of tested species, especially for cephalexin and sulfamethoxazole. The number of current tested species of cephalexin and sulfamethoxazole for MIC were 14 and 12 in EUCAST database, representatively, and the smaller number (<30) of tested species would result in the overestimation on the MIC (Bengtsson-Palme and Larson, 2016). Even an adjusted factor was applied depending on the number of tested species, it still might overestimate the results due to the intra-species bias variability in sensibility. In the future work, more tested species should be applied to verify the results.

Table 6-1 Estimated minimum selective concentrations (MSCs, μg/L) of target antibiotics.

Antibiotic	Number of tested species[1]	Lowest MIC[2] (μg/L)	Size-adjusted lowest MIC[3] (μg/L)	Estimated MSC (μg/L)
Amoxicillin	46	4	4	0.400
Ampicillin	79	4	4	0.400
Piperacillin	43	8	4	0.400
Cefazolin	21	30	15.366	1.537
Cephalexin	14	250	85.366	8.537
Cefaclor	11	16	4.293	0.429
Ceftriaxone	44	2	2	0.200
Cefdinir	5	30	3.659	0.366
Meropenem	78	2	2	0.200
Clarithromycin	21	4	2.049	0.205
Azithromycin	20	8	3.902	0.390
Levofloxacin	59	4	4	0.400
Sulfamethoxazole	11	1000	268.293	26.829
Trimethoprim	34	8	6.634	0.663

[1] Data from the EUCAST MIC distribution website, last accessed 2021-09-21(http://www.eucast.org).

[2] The lowest MICs with at least 10 observations at this concentration.

[3] Calculated by the formula in Bengtsson-Palme and Larsson (2016): [Observed lowest MIC] * [number of tested species]/41, where 41 is a constant determined from the resampling data.

6.3.3 Comparison of PNEC between ecotoxicity and AMR selection

The $PNEC_R$ for the AMR selection was calculated based on updated size-adjusted MIC of antibiotics according to Bengtsson-Palme and Larson (2016) and compared with the traditional ERA $PNEC_{sw}$ for the ecotoxicity (Figure 6-3). Since there was no ecotoxicity data for piperacillin, cefaclor and cefdinir, the $PNEC_R$ of these compounds was directly taken for further risk characterization. Generally, the $PNEC_{sw}$ of target antibiotics shows a larger range from 19 ng/L to 100 µg/L than $PNEC_R$ (20 ng/L to 2683 ng/L). The $PNEC_{sw}$ value of ampicillin, azithromycin, cephalexin and sulfamethoxazole were lower than those of $PNEC_R$, while the $PNEC_R$ of other compounds were lower. For cephalexin and sulfamethoxazole, the $PNEC_R$ are much greater compared to other antibiotics, which might be caused by the overestimation of $PNEC_R$ as discussed above.

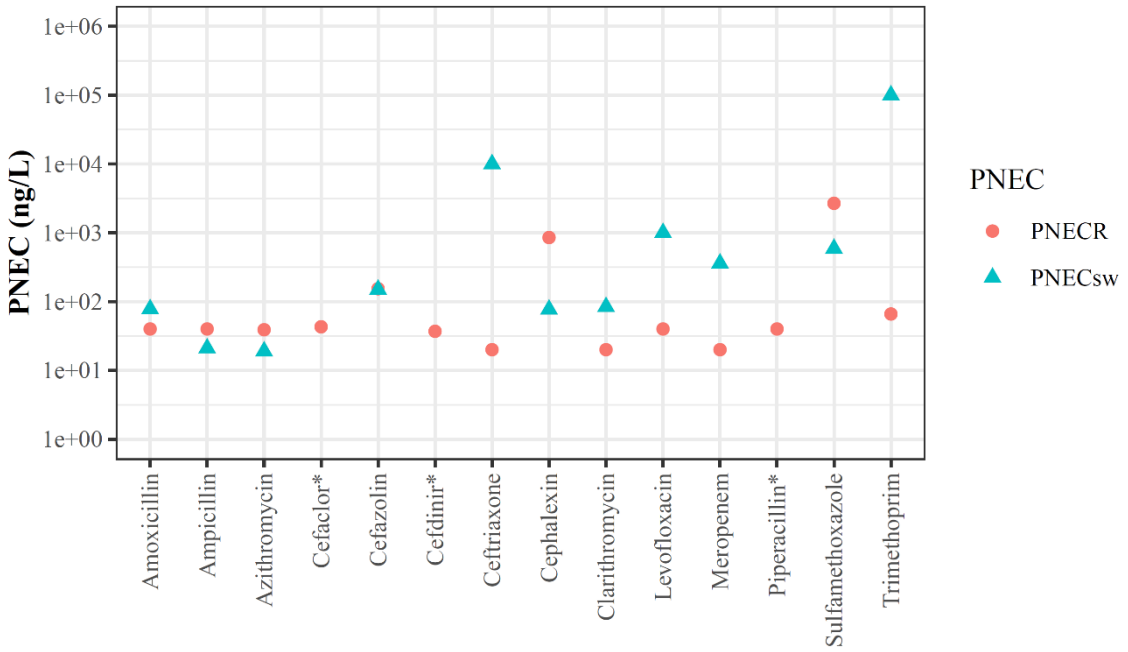


Figure 6-2 Comparison of PNEC calculated by ecotoxicity ($PNEC_{sw}$) and AMR selection ($PNEC_R$). *: ecotoxicity data was not available.

From the view of comparison, it is essential to apply the same or similar endpoint. Le Page et al. (2017) also compared the PNEC based on the MIC of CRB and NOEC of cyanobacteria and found that the sensitivity of CRB and cyanobacteria were not significantly different. However, the author applied different endpoints to calculate the PNEC between CRB and cyanobacteria, which results in the underestimation of the

sensibility of CRB. Based on the growth curve (Figure 6-2), MIC represents the minimum inhibitory concentration with the growth rate of zero, however, the NOEC stands for the minimum concentration with no inhibition on growth rate. Le Page et al. (2017) directly took the theoretical $PNEC_R$(T) from Bengtsson-Palme and Larsson (2016) for some antibiotics, while the $PNEC_R$(T) represents the theoretical MSCs of antibiotics in Bengtsson-Palme and Larsson (2016).

Table 6-2 Comparison on PNEC between ecotoxicity and AMR selection and lower PNEC ($PNEC_L$) applied for risk characterization

Antibiotic	**$PNEC_R$ (ng/L)**	**$PNEC_{sw}$ (ng/L)**	**$PNEC_L$ (ng/L)**
Amoxicillin	40	78	40
Ampicillin	40	21	21
Piperacillin*	40	-	40
Cefazolin	154	150	150
Cephalexin	854	77	77
Cefaclor*	43	-	43
Ceftriaxone	20	10,000	20
Cefdinir*	37	-	37
Meropenem	20	360	20
Clarithromycin	20	84	20
Azithromycin	39	19	19
Levofloxacin	40	1,000	40
Sulfamethoxazole	2683	590	590
Trimethoprim	66	100,000	66

*: the ecotoxicity test data was not available, $PNEC_R$ was directly taken as $PNEC_L$.

-: not available.

During the calculation of PNEC, an assessment factor (AF) of 10 was applied in traditional ERA, the AF is an expression of uncertainty in the extrapolation from a limited number of tested species to complex ecosystems in the actual environment, and also explains the interspecies and differences of sensibility and extrapolation from laboratory data to field impact (EAMA, 2018). Even the same AF was applied when we calculate the $PNEC_R$ from estimated MSC, the value of 10 is still needed to be further

judged. Furthermore, a size-adjusted factor has already been applied depending on the number of tested species to adjust the lowest MIC for the target antibiotics, which means that we have considered the inter- and intra- species variability of sensitivity to antibiotics. However, for the risk assessment and the proposal of discharge limits, the PNEC level should be strict to fulfill the protective goal, hence we considered it acceptable for the current calculation strategy, and the lower PNEC ($PNEC_L$) between $PNEC_R$ and $PNEC_{sw}$ would be applied for the risk assessment (Table 6-2). It is worthy to be noticed that both $PNEC_R$ and $PNEC_{sw}$ of sulfamethoxazole might be overestimated due to the small number of tested species during both traditional ecotoxicity test and MIC test, which results in that the $PNEC_L$ of sulfamethoxazole could not fulfill the protection of environmental and human health.

6.3.4 Risk assessment and discharge limits

During the estimation on the removal of antibiotics in STPs, the worst-scenario of predicted environmental concentration in secondary effluent (PEC_{sec_eff}) of antibiotics were assessed. The PEC_{sec_eff} of the two target STP and $PNEC_L$ were applied to characterize the potential risk for the worst-scenario, and the related risk quotients (RQs) are given in Figure 6-3. Generally, the RQ values for STP A are lower than those of STP B due to the higher removal of antibiotics. For the PEC_{sec_eff} of STP A, amoxicillin, ampicillin, piperacillin, clarithromycin, azithromycin and levofloxacin showed high risk with an average RQ value of 2.91, 1.17, 3.84, 22.43, 10.61 and 13.58, respectively. Since the β-lactams (amoxicillin, ampicillin and piperacillin) can be easily hydrolyzed (Mayers, 2009), the risk of these compounds might be overestimated. These above six antibiotics also showed high risk in STP B, with an average RQ value of 3.26, 1.07, 8.15, 36.30, 22.32 and 26.68, respectively. The average RQ value of clarithromycin, azithromycin and levofloxacin were all above 10 for the two target STPs, which should be paid more attention. The average RQ value of ceftriaxone, meropenem and trimethoprim in STP A was below 1, while they showed high risk (average RQ = 3.38, 2.23 and 1.33, respectively) in STP B. For sulfamethoxazole, although the RQ value was below 1 for both STPs, more tested species should be

applied for further ecotoxicity and MIC test to recorrect the PNEC applied for risk assessment.

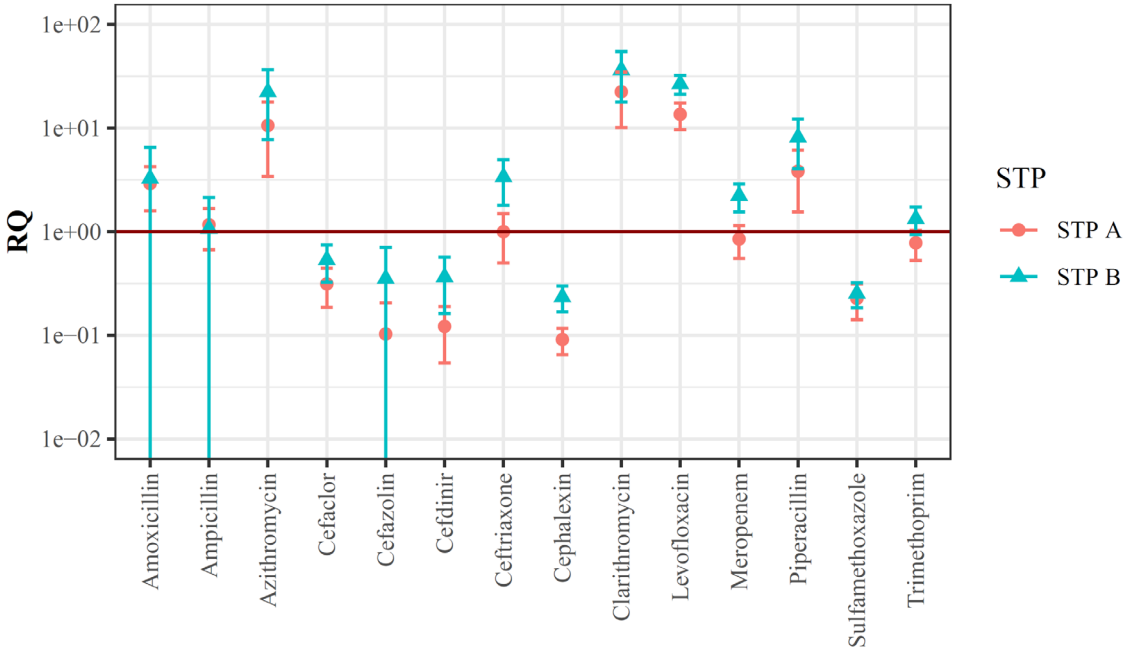


Figure 6-3 Risk characterization by risk quotient (RQ) for the predicted environmental concentration of secondary effluent (PEC_{sec_eff}) in the two target STPs. Red line: RQ = 1; the point in the figure is the average value of RQ, and the upper and bottom line shows the maximum and minimum value of RQ for the worst-scenario.

To propose the discharge limit, it is important to clarify our protection goals. In this study, the sensibility of target antibiotics among traditional ERA species were compared and the NOEC or EC10s of most sensitive species was selected for risk assessment, which is to fulfill to goal of protecting the microbial diversity and functions of ecosystem (environmental protection). Furthermore, the lowest size-adjusted MIC of target antibiotics in the EUCAST database was selected to estimate the MSC for the further $PNEC_R$ calculation, which is targeted to protect human health by avoiding the AMR selection. Then the lower PNEC between $PNEC_{sw}$ and $PNEC_R$ was applied for risk assessment by considering both protective goals of environmental and human health. Le Page et al. (2017) proposed one single value (100 ng/L) of discharge limit for all antibiotics by determining the 5th percentile for growth inhibition data for cyanobacteria and environmental bacteria and MICs for CRB in order to protect the bacterial NOECs with 95% confidence. However, no matter in our study or other previous studies, the estimated PNEC based either ecotoxicity or MICs varied seriously,

one single value is not explicit enough to make balance between the costs deriving from the demand of removal enhancement and the protection goals, which is coincident with Bengtsson-Palme et al. (2018). Therefore, the separate discharge limits ($PNEC_L$ in Table 6-2) for different antibiotics are proposed in this study for protection environmental and human health.

There are still some limitations of the proposed discharge limits. Either the ecotoxicity data or the MIC data was based on single compound to single species, which might be far from the actual situation (mixture of antibiotics to complex community). There might be both synergistic and antagonistic effects for the mixture of antibiotics on the traditional ERA tested species (Wang et al., 2018; Yang et al., 2021), while it may result in lower MSCs when the CRB were exposed to the mixture of antibiotics (Gullberg et al., 2014). Moreover, a new experiment by Wood (2019) showed that the complex natural microbial communities can hamper the resistance selection through improving the fitness cost or providing protection to susceptible strains, which is coincident with Klümper et al. (2019). Hence, to make the proposed discharge limits closer to our protection goals, further studies are required to test the ecotoxicity or MICs of mixtures of antibiotics on the natural microbial communities.

6.3.5 Reduction strategy of the antibiotics discharge from STPs

As discussed above, PEC_{sec_eff} of six antibiotics in STP A are above the discharge limit, which are amoxicillin, ampicillin, piperacillin, clarithromycin, azithromycin and levofloxacin; while besides these compounds, PEC_{sec_eff} of three more antibiotics (ceftriaxone, meropenem and trimethoprim) in STP B cannot fulfill the discharge limit. To achieve the discharge goal, there are three possible strategies to reduce the discharge volumes of these antibiotics: 1) consumption reduction; 2) enhancement on the removal of antibiotics during secondary treatment; 3) further advanced treatment for the secondary effluent. When a single strategy is applied, the percentage of consumption reduction, secondary treatment removal enhancement and further treatment for secondary effluent of each compound are given in Table 6-3.

If only consumption reduction strategy was applied, except for ampicillin consumption in Prefecture S (STP A location) (13%) and F (STP B location) (17%) and

trimethoprim consumption in Prefecture F (25%), the reduction percentage of other compounds should be higher than 50%, the reduction of clarithromycin, azithromycin and levofloxacin even should be above 90%. For the consumption reduction, Japanese government also reported a reduction target by considering the requirement of clinical AMR situation, the reported action performances from 2014 to 2019 and target in 2020 for the human-antibiotics consumption are shown in Table 6-4. The reduction target of oral cephalosporins (including ceftriaxone), macrolides (including clarithromycin and azithromycin), and fluoroquinolones (including levofloxacin) are all 50% to 2020, however, it is reasonable to doubt if the target can be achieved considering the reduction trends from 2014 to 2019. Even the target for the intravenous antibiotics is 20% reduction to 2020, the consumption data from 2014 to 2019 shows an increasing trend. There is no specific target for oral penicillins and combinations of sulfonamides and trimethoprim, however, as the trends in Table 6-3 shown, the consumption of oral penicillins (including amoxicillin, ampicillin and piperacillin) and combinations of sulfonamides and trimethoprim (mainly including sulfamethoxazole and trimethoprim) increased from 2013 to 2019, with a proportion of 28.4% and 52.0%, respectively. Therefore, no matter the current consumption performances or the action plan of the consumption reduction for clinical AMR issue could not fulfill the discharge goal of antibiotics from STPs. Even more strict targets were set, according to the consumption trends shown in Table 6-3, it is difficult to achieve in recent years due to the requirement of antibiotics for infection treatment. Hence, it is necessary to combine other strategies with the consumption reduction.

The second strategy is to improve the removal efficiency of antibiotics during secondary treatment. For most antibiotics, the removal efficiencies during secondary treatment in AAO process (STP A) are higher than those in CAS (STP B) (Table 5-13), hence the AAO process could be an alternative for the performance enhancement of CAS. Even though there are still six compounds (amoxicillin, ampicillin, piperacillin, clarithromycin, azithromycin and levofloxacin) in the secondary effluent of STP A above the discharge limits. Advance biological treatments (e.g., Aerobic-MBR, AO-MBR, AAO-MBR and so on) by combining the activated sludge system with membrane have been verified to enhance the removal of antibiotics (Schröder et al., 2012; Sipma et

al., 2010; Tran et al., 2016; Zhu et al., 2017). Adsorption could be improved by the higher MLSS concentration (Sipma et al., 2010), and longer SRT can increase the microbial diversity by allowing the enrichment of slow-growing bacteria (e.g., AOB) to enhance the biodegradation of antibiotics (Tran et al., 2016), which could overall enhance the removal of antibiotics. However, even the advanced biological treatments were applied, the removal of antibiotics can only be moderately enhanced. For the β-lactams (amoxicillin, ampicillin, piperacillin, ceftriaxone and meropenem) which call for 8-33% enhancement on the removal, the advanced biological treatments might help to achieve the discharge goal; while for clarithromycin, azithromycin and levofloxacin, more effective technology are required to fulfill the need of large enhancement (>100%). Moreover, higher operating cost for the membrane and membrane fouling issue should also be considered.

Table 6-3 Three reduction strategies to achieve the discharge limit for the two target STPs (the percentage shown represents the amount that the single strategy applied)

Antibiotic	Discharge limit (ng/L)	Strategy to achieve the discharge goal					
		Consumption reduction (%)		Secondary treatment enhancement (%)		Further treatment for secondary effluent (%)	
		STP A	STP B	STP A	STP B	STP A	STP B
Amoxicillin	40	66%	69%	8%	7%	7%	7%
Ampicillin	21	13%	7%	2%	1%	1%	1%
Piperacillin	40	74%	88%	33%	91%	23%	45%
Ceftriaxone	20	-	70%	-	22%	-	17%
Meropenem	20	-	55%	-	16%	-	13%
Clarithromycin	20	96%	97%	114%	322%	52%	75%
Azithromycin	19	91%	96%	116%	3357%	51%	93%
Levofloxacin	40	93%	96%	224%	625%	66%	83%
Trimethoprim	66	-	25%	-	2386%	-	25%

-: the discharged concentrations of secondary effluent were below the discharge limit.

As the requirement of further treatment, the third strategy (advanced treatment for secondary effluent) becomes a promising approach. The removal of antibiotics by sand filter (STP A) and chlorination disinfection (STP B) after secondary treatment were investigated in Chapter 5 and the results are shown in 5.3.2. Chlorination showed little

removal of antibiotics, and a little higher removal by sand filter (levofloxacin-17.2%; clarithromycin-4.5%; azithromycin-2.2%) was highly depended on the sorption distribution coefficient of antibiotics, which means the removal by sand filter was attributed to adsorption. However, their removal by either chlorination disinfection or sand filter was not sufficient to achieve the discharge target and more effective technology is needed.

Table 6-4 National action performances (2014-2019 compared to 2013) and plan (2020) for human-used antibiotics consumption in Japan (Ministry of Health, Labour, Welfare of Japan, 2019)

	Consumption in 2013 (DID*)	Compared to 2013							
		average of 2014-2016[#]	2014	2015	2016	2017	2018	2019	Target in 2020
Oral penicillins	0.88	8.0% increase	1.1% increase	12.5% increase	10.2% increase	8.0% increase	14.8% increase	28.4% increase	-
Oral cephalosporins	3.91	3.7% reduction	3.3% reduction	2.1% reduction	5.6% reduction	14.2% reduction	18.4% reduction	22.7% reduction	50.0% reduction
Oral macrolides	2.82	5.8% reduction	6.8% reduction	5.0% reduction	5.6% reduction	13.5% reduction	18.0% reduction	20.6% reduction	50.0% reduction
Oral fluoroquinolones	4.83	2.0% reduction	0.4% increase	3.9% reduction	2.5% reduction	9.1% reduction	17.0% reduction	18.1% reduction	50.0% reduction
Oral combinations of sulfonamides and trimethoprim	0.25	16.0% increase	8.0% increase	16.0% increase	24.0% increase	32.0% increase	44.0% increase	52.0% increase	-
Intravenous antibiotics	0.96	3.8% increase	No change	4.2% increase	7.3% increase	4.1% increase	10.0% increase	12.7% increase	20.0% reduction

-: no target;

*: defined daily dose per 1,000 inhabitants per day;

[#]: since the strategy in consumption data application (3.2.4), the average consumption volume from 2014 to 2016 for some antibiotics were applied for the prediction, here the average performances from 2014 to 2016 are provided.

In recent years, the additional physical-chemical treatment technology (e.g., activated carbon adsorption, membrane and advanced oxidation process) to treat antibiotics in secondary effluent become more popular. Among the membrane technologies, reverse osmosis (RO) and nanofiltration (NF) showed higher application prospects due to high removal efficiency on micropollutants and relatively low initial investment (Acero et al., 2010; Liu et al., 2014). Watkinson et al. (2007) investigated

the plant with microfiltration/reverse osmosis (MF/RO) process to treat CAS effluent and found that microfiltration removed around 43% of total antibiotics from the liquid phase, and almost all β-lactam antibiotics can be removed; the RO membrane reduced 94% of the RO-feed concentration of antibiotics. Acero et al. (2010) compared four ultrafiltration (UF) membranes and four NF membranes to treat the secondary effluent and found highest eliminations (>75%) for all pharmaceutical compounds in the case of NF with HL membrane. However, the main issues of the membrane technologies application in STPs are membrane fouling and the disposal of concentrate. The advanced oxidation processes (AOPs), such as ozonation, fenton, photocatalytic, and electrochemical oxidation, have been reported to effectively remove the micropollutants in the membrane concentrate and secondary effluent as well (Pérez-González et al., 2012; Wang and Zhuan, 2020). Liu et al. (2014) did a systematic study on the removal of antibiotics by NF combined with AOPs (UV_{254} photolysis, ozonation and UV/O_3 process) for NF concentrate treatment, and high rejections (>98%) of antibiotics were reported of NF, ozone-based processes (ozonation and UV/O_3 process) showed high removal efficiencies (>87%) in 30 min. Nakada et al. (2007) investigated the removal of pharmaceuticals (including clarithromycin, azithromycin, sulfamethoxazole and trimethoprim) by ozonation in a municipal STP and also obtained high removal efficiency (80%) for antibiotics. Although AOPs show high removal efficiency for antibiotics, they exhibited their own advantages and disadvantages for different processes on operating conditions, cost, utilization efficiency, secondary pollutants, etc. (Wang and Zhuan, 2020), which should be further considered during the application.

In summary, since the increasing trends for the consumption of penicillins (including amoxicillin, ampicillin and piperacillin), cephalosporins (including ceftriaxone) and combinations of sulfonamides and trimethoprim, the consumption reduction strategy is difficult to achieve, while either secondary treatment enhancement strategy through advanced biological treatment or further treatment for secondary treatment strategy can help to fulfill the discharge target for these compounds. However, for clarithromycin, azithromycin and levofloxacin, even the current decreasing consumption trend and the moderately effective enhancement by advanced biological treatment are both considered, it cannot totally achieve the discharge limit. The further

treatment of secondary effluent is necessary for these compounds.

6.4 Conclusions

Risk assessment for the PEC_{sec_eff} of antibiotics in Chapter 5 was carried out by considering both environmental (ecotoxicity) and human health (AMR selection issue) perspectives. On the basis of risk assessment, the discharge limit of individual antibiotic from the STPs and the possible strategies for the reduction of released antibiotics above the discharge limit were proposed. The major findings from this Chapter were as follows:

- ✓ In the traditional ecotoxicity tests, cyanobacteria are the most sensitive for β-lactams (amoxicillin, ampicillin, cefazolin, ceftriaxone, cephalexin and meropenem) and macrolides (azithromycin and clarithromycin), while *Lemna gibba* (macrophytes) is the most sensitive species for levofloxacin and trimethoprim, more sensitive species should be considered for sulfonamides (including sulfamethoxazole).
- ✓ The $PNEC_{sw}$ (calculated by the ecotoxicity data) of target antibiotics shows a larger range from 19 ng/L to 100 μg/L than $PNEC_R$ (calculated by AMR selection data) (20 ng/L to 2683 ng/L). The $PNEC_{sw}$ value of ampicillin, azithromycin, cephalexin and sulfamethoxazole were lower than those of $PNEC_R$, while the $PNEC_R$ of other compounds were lower. However, the standard of the $PNEC_R$ calculation should be established by considering both the growth inhibition curve and assessment factor.
- ✓ The RQ values for STP A are lower than those of STP B due to the higher removal of antibiotics. For the PEC_{sec_eff} of STP A, amoxicillin, ampicillin, piperacillin, clarithromycin, azithromycin and levofloxacin showed high risk with an average RQ value of 2.91, 1.17, 3.84, 22.43, 10.61 and 13.58, respectively. These above six antibiotics also showed high risk in STP B, with an average RQ value of 3.26, 1.07, 8.15, 36.30, 22.32 and 26.68, respectively. The average RQ value of ceftriaxone, meropenem and trimethoprim in STP A was below 1, while they showed high risk (average RQ = 3.38, 2.23 and 1.33, respectively) in STP B.
- ✓ PEC_{sec_eff} of six antibiotics in STP A are above the discharge limit, which are amoxicillin, ampicillin, piperacillin, clarithromycin, azithromycin and levofloxacin;

while besides these compounds, PEC_{sec_eff} of three more antibiotics (ceftriaxone, meropenem and trimethoprim) in STP B cannot fulfill the discharge limit.

- ✓ For amoxicillin, ampicillin, piperacillin, ceftriaxone, meropenem, and trimethoprim, either secondary treatment enhancement strategy through advanced biological treatment or further treatment for secondary treatment strategy can help to fulfill the discharge target for these compounds; for clarithromycin, azithromycin and levofloxacin, the combinations of consumption reduction, secondary treatment enhancement and further treatment of secondary effluent should be considered.

6.5 Reference

Acero, J. L., Benitez, F. J., Leal, A. I., Real, F. J., Teva, F., 2010. Membrane filtration technologies applied to municipal secondary effluents for potential reuse. *Journal of Hazardous Materials*, *177*(1-3), pp.390-398.

Bengtsson-Palme, J., Larsson, D. J., 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. *Environment International*, *86*, pp.140-149.

Bengtsson-Palme, J., Larsson, D. J., 2018. Protection goals must guide risk assessment for antibiotics. *Environment International*, *111*, pp.352-353.

Brandt, K. K., Amézquita, A., Backhaus, T., Boxall, A., Coors, A., Heberer, T., Lawrence, J. R., Lazorchak, J., Schönfeld, J., Snape, J. R., Zhu, Y., Topp, E., 2015. Ecotoxicological assessment of antibiotics: a call for improved consideration of microorganisms. Environment International, 85, pp.189-205.

EMEA, 2018. Guideline on the environmental risk assessment of medicinal products for human use (draft). Available from: https://www.ema.europa.eu/documents/scientific-guideline/draft-guideline-environmental-risk-assessment-medicinal-products-human-use-revision-1_en.pdf (accessed 25 June 2019).

Gullberg, E., Albrecht, L. M., Karlsson, C., Sandegren, L., Andersson, D. I., 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. *MBio*, *5*(5), e01918-14.

Gullberg, E., Cao, S., Berg, O. G., Ilbäck, C., Sandegren, L., Hughes, D., Andersson, D. I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathogens, 7*(7), pp1002158.

Klümper, U., Recker, M., Zhang, L., Yin, X., Zhang, T., Buckling, A., Gaze, W. H., 2019. Selection for antimicrobial resistance is reduced when embedded in a natural microbial community. *The ISME Journal*, *13*(12), pp.2927-2937.

Le Page, G., Gunnarsson, L., Snape, J., Tyler, C. R., 2017. Integrating human and environmental health in antibiotic risk assessment: a critical analysis of protection goals, species sensitivity and antimicrobial resistance. *Environment International*, *109*, pp.155-169.

Liu, A., Fong, A., Becket, E., Yuan, J., Tamae, C., Medrano, L., ... Miller, J. H., 2011. Selective advantage of resistant strains at trace levels of antibiotics: a simple and ultrasensitive color test for detection of antibiotics and genotoxic agents. *Antimicrobial Agents and Chemotherapy*, *55*(3), pp.1204-1210.

Liu, P., Zhang, H., Feng, Y., Yang, F., Zhang, J. 2014. Removal of trace antibiotics from wastewater: a systematic study of nanofiltration combined with ozone-based advanced oxidation processes. *Chemical Engineering Journal*, *240*, pp.211-220.

Mayers, D.L. ed., 2009. *Antimicrobial drug resistance* (pp. 803-824). New York: Humana press.

Ministry of Health, Labour, and Welfare of Japan, 2019. Nippon AMR One Health Report (NAOR) 2019. https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000120172.html

Nakada, N., Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N., Takada, H., 2007. Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant. *Water Research*, *41*(19), pp.4373-4382.

OECD, 2010. OECD. Test No. 209: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation) ed^eds. *OECD Publishing* (2010).

Pérez-González, A., Urtiaga, A. M., Ibáñez, R., Ortiz, I., 2012. State of the art and review on the treatment technologies of water reverse osmosis concentrates. *Water Research*, *46*(2), pp.267-283.

Sandegren, L., 2019. Low sub-minimal inhibitory concentrations of antibiotics generate new types of resistance. *Sustainable Chemistry and Pharmacy*, *11*, pp.46-48.

Schröder, H.F., Tambosi, J.L., Sena, R.F., Moreira, R.F.P.M., José, H.J., Pinnekamp, J., 2012. The removal and degradation of pharmaceutical compounds during membrane bioreactor treatment. *Water Science and Technology*, *65*(5), pp.833-839.

Sipma, J., Osuna, B., Collado, N., Monclús, H., Ferrero, G., Comas, J., Rodriguez-Roda, I., 2010. Comparison of removal of pharmaceuticals in MBR and activated sludge systems. *Desalination*, *250*(2), pp.653-659.

Tran, N.H., Chen, H., Reinhard, M., Mao, F., Gin, K.Y.H., 2016. Occurrence and removal of multiple classes of antibiotics and antimicrobial agents in biological wastewater treatment processes. *Water Research*, *104*, pp.461-472.

von der Ohe, P. C., Dulio, V., Slobodnik, J., De Deckere, E., Kühne, R., Ebert, R. U., Ginebreda, A., Cooman, W. D., Schüürmann, G., Brack, W., 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European Water Framework Directive. *Science of the Total Environment*, *409*(11), pp.2064-2077.

Wang, D., Wu, X., Lin, Z., Ding, Y., 2018. A comparative study on the binary and ternary mixture toxicity of antibiotics towards three bacteria based on QSAR investigation. *Environmental Research*, *162*, pp.127-134.

Wang, J., Zhuan, R., 2020. Degradation of antibiotics by advanced oxidation processes: An overview. *Science of the Total Environment*, *701*, pp.135023.

Watkinson, A. J., Murby, E. J., Costanzo, S. D., 2007. Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. *Water Research*, *41*(18), pp.4164-4176.

Wess, R.A, Schmidt, T., Höger, S., 2020. Challenges of Regulatory Environmental Risk Assessment for Human Pharmaceuticals with Focus on Antibiotics. *Chimia (Aarau), 74*(3), pp.183-191.

Westhoff, S., van Leeuwe, T. M., Qachach, O., Zhang, Z., van Wezel, G. P., Rozen, D. E., 2017. The evolution of no-cost resistance at sub-MIC concentrations of streptomycin in Streptomyces coelicolor. *The ISME journal*, *11*(5), pp.1168-1178.

Wood, K., 2019. Microbial ecology: complex bacterial communities reduce selection for antibiotic resistance. *Current Biology*, *29*(21), pp.1143-1145.

World Health Organization, 2014. Antimicrobial Resistance: 2014 Global Report on Surveillance ed^eds. *World Health Organization* (2014).

Yang, L., Feng, Y. X., Zhang, H., Yu, X. Z., 2021. Estimating the synergistic and antagonistic effects of dual antibiotics on plants through root elongation test. *Ecotoxicology*, *30*(8), pp.1598-1609.

Zhu, Y., Wang, Y., Jiang, X., Zhou, S., Wu, M., Pan, M., Chen, H., 2017. Microbial community compositional analysis for membrane bioreactor treating antibiotics containing wastewater. *Chemical Engineering Journal*, *325*, pp.300-309.

Chapter VII
Conclusions and Recommendations

7.1 Summary of Conclusions

Since the massive and continuous usage of antibiotics in our everyday life, it has raised worldwide concerns due to the ecotoxicity in the aquatic environment and development of antibiotic resistant. For the further risk assessment on the antibiotics in the aquatic environment, it is essential to evaluated the discharge of antibiotics from the sewage treatment plants (STPs). The occurrences of antibiotics exhibit great variability influenced by several factors, such as types, locations, seasons and so on. Moreover, monitoring of antibiotics is restricted by the available analytical methods in laboratories, cost and time consumption. Therefore, estimation approach becomes a promising way. Because the discharge of STPs is considered as the main route for human-used antibiotics entering into the aquatic environment, to evaluate the discharge of antibiotics from STPs, it involved two main parts to establish the predictive models: I) to predict the antibiotics concentrations in the sewage influent based on the consumption volume, wastewater production per inhabitant and service population of target STPs; II) to predict the concentration of antibiotics in the STPs effluent based on the removal performance of antibiotics in the STPs. In this study, two parts of predictive models were established and evaluated by the measured data separately, the possible strategy was obtained for the further assessment. On the basis of these prediction results, risk assessment was carried out for the antibiotics discharged from STPs by considering both environmental (ecotoxicity) and human health (AMR issue) perspectives. Furthermore, the discharge limit of individual antibiotic from the STPs and the possible strategies for the reduction of released antibiotics above the discharge limit were proposed. The findings obtained in each Chapter are summarized as below.

In Chapter III, predicting equation based on consumption volumes of human-used antibiotics from two databases (shipping and prescription) had been applied to estimate the sewage influent concentrations of selected antibiotics in two STPs which located on different prefectures, and monitoring data (6 times between May,2019 and March, 2020) were used to evaluate the accuracy of this predicting equation. Nine target compounds were measured in the sewage influent of two STPs. In STP A, the concentrations of target antibiotics in influents were ranged from 28 ng/L (ampicillin) to 1235 ng/L (levofloxacin), while in STP B, it was ranged from 35 ng/L (cefazolin) to 1471 ng/L (levofloxacin), and the seasonal fluctuations in STP B were relatively larger than that of STP A. In two STPs, among the 9 detected antibiotics, 7 have a $PECs_{inf}$ calculated by national shipping volume greater than the corresponding $MECs_{inf}$, and 6 have a $PECs_{inf}$ calculated based on national/regional prescription volume greater than the corresponding $MECs_{inf}$. The $PECs_{inf}$ on the basis of prescription volumes are closer to $MECs_{inf}$ than those on the basis of shipping volume, but the predicted concentrations of azithromycin based on the prescription volumes were unacceptably low, which caused by human error of the NDB database. There were positive correlations between national shipping and national/regional prescription databases (correlation efficient r>0.70). Therefore, it is possible to use the national shipping volumes to calculate the predicted concentrations when the human error happened on the regional prescription data of target compounds, but the predicted concentrations would be somewhat higher than those on the basis of national shipping data. The strategy in this part could be obtained: the $PECs_{inf}$ calculated by the regional prescription data would be applied for further estimation, for the compound with large statistical error, the consumption volume could be revised on the basis of national shipping volumes.

In Chapter IV, the batch experiments were carried out to study the adsorption and biodegradation performance of target antibiotics in activated sludge processes from AAO, CAS and MBR system of the two target STPs for the further estimation on the removal in target STPs. For the sorption distribution coefficients (K_d), the empirical predictive model was established and evaluated by the measured data. The biodegradation of target antibiotics was estimated by two kinetics: 1) first-order kinetics; 2) separately characterization on AOB co-metabolic kinetics and heterotroph

biodegradation kinetics (first-order kinetics). All β-lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin), sulfamethoxazole and trimethoprim were classified as lowly sorptive (log K_d < 2). The K_d values of β-lactams (amoxicillin, ampicillin, piperacillin and cefazolin) ranged from 2.4 to 28.4 L/kg (log K_d =0.39-1.45), the average log K_d value (n=5) of these four compounds for all redox conditions and all STPs were 1.00 ± 0.12, 0.98 ± 0.13, 0.83 ± 0.06, and 0.66 ± 0.12, respectively. Clarithromycin and azithromycin were classified as moderately sorptive (2 < log K_d < 3). The K_d values for clarithromycin ranged from 236 to 1315 L/kg (log K_d =2.73 ± 0.13, n=5). Levofloxacin was the only compound classified as highly sorptive (log K_d > 3) in this study, the K_d values ranged from 272 to 6484 L/kg (log K_d =3.00 ± 0.34, n=5). The predictive model of sorption distribution coefficient in this study was established and would be applied in further estimation in Chapter V: log K_{oc}= 0.63 log K_{ow} + 1.15, K_d = $f_{oc}K_{oc}$, f_{oc}=0.531, which generally shows good prediction for most compounds with RMSE=0.47. However, the sorption distribution coefficients of levofloxacin were underestimated in all predictive models based on the log K_{ow} values, then the experimental data of levofloxacin would be applied in the further estimation. The first-order kinetic constants (k_{bio}, $L \cdot gVSS^{-1} \cdot d^{-1}$) were estimated on the biodegradation of target antibiotics in the activated sludge from three different redox conditions in STP A_AAO, and aerobic condition in STP B_CAS and STP B_MBR. B-Lactam antibiotics (amoxicillin, ampicillin, piperacillin and cefazolin) were highly (1 < k_{bio} < 5) or very highly biodegradable (k_{bio} > 5) under three redox conditions and all sludge sources. Sulfamethoxazole and levofloxacin were hardly biodegradable under all redox conditions and sludge sources (k_{bio} <0.5 L/gVSS-d). Clarithromycin was highly biodegradable in STP B_MBR and STP A_Aerobic sludge, while it was moderately biodegradable under STP B_CAS sludge and hardly biodegradable under STP A_Anoxic and STP A_Anaerobic condition. Azithromycin was highly biodegradable by STP B_MBR sludge, moderately biodegradable under STP A_Aerobic condition, while it was hardly biodegradable under anoxic condition and by STP B_CAS sludge, and no degradation under anaerobic condition. The biodegradable abilities of trimethoprim were much higher in anoxic and anaerobic conditions (k_{bio} >1) than those in aerobic conditions (k_{bio} < 0.5). Separately estimation and characterization on the contribution of

AOB co-metabolism could help to better understand the mechanisms of biodegradation of each antibiotic in activated sludge, however, the estimation accuracy was higher for first-order kinetics due to the one-time estimation. Therefore, the first-order biodegradation rate constants of target antibiotics would be applied for further estimation in Chapter V.

In Chapter V, the integration of the primary and secondary treatment models was applied to estimate the fate of antibiotics in the STPs based on the estimated K_d and k_{bio} value in Chapter IV, and the investigation on the fate of target antibiotics were also carried out in the two target STPs. After evaluate the results by measured data, a strategy for the prediction of antibiotics concentration in secondary effluent of target STPs was achieved and applied to obtain the PEC_{sec_eff}. All the target antibiotics can be detected in influent and primary effluent. The concentrations of amoxicillin, ampicillin and cefazolin in secondary of effluent and effluent were below the limits of detection (LODs) in the two STPs. In STP A, the highest average concentration of target antibiotics detected in the effluent was for sulfamethoxazole (263 ng/L), and the concentrations of other compounds detected in the effluent were all lower than 100 ng/L. In STP B, the concentrations of all target compounds in MBR-effluent were lower than those in CAS-effluent. The estimated removal of antibiotics by wasting primary and excess sludge were acceptable, while for the biodegradation, the accuracy of predicted removal for the moderately and hardly biodegradable antibiotics were relatively low, which was mainly resulted from the lower estimated accuracy of k_{bio} in Chapter IV. A fifteen-group classification on the estimated removal efficiency based on the K_d value and k_{bio} value of antibiotics was established and applied to obtain the PEC_{sec_eff} of antibiotics in target STPs. In general, the PEC_{sec_eff} of antibiotics in STP A were lower than that in STP B due to the higher wastewater production per inhabitant per day and higher removal efficiency in STP A. The discharge of β-lactams was relatively low even the total consumption was high in Japan, which was resulted from the highly degradable character. For the antibiotics from other class, even the removal in AAO system were higher than that in CAS system, the PEC_{sec_eff} from the target STP A were still high, which require further advanced treatment showed higher removal of antibiotics, e.g., ozonation. As the results in this study, MBR could also improve the biodegradation

ability or shorten the half-lives of antibiotics due to the higher microbial diversity and MLSS, which could be considered an alternative for the further STP upgrading.

In Chapter VI, the PEC_{sec_eff} of antibiotics from the two STPs were applied for the risk assessment. The $PNEC_{sw}$ (calculated by the ecotoxicity data) of target antibiotics shows a larger range from 19 ng/L to 100 μg/L than $PNEC_R$ (calculated by AMR selection data) (20 ng/L to 2683 ng/L). The $PNEC_{sw}$ value of ampicillin, azithromycin, cephalexin and sulfamethoxazole were lower than those of $PNEC_R$, while the $PNEC_R$ of other compounds were lower. The RQ values for STP A are lower than those of STP B due to the higher removal of antibiotics. For the PEC_{sec_eff} of STP A, amoxicillin, ampicillin, piperacillin, clarithromycin, azithromycin and levofloxacin showed high risk with an average RQ value of 2.91, 1.17, 3.84, 22.43, 10.61 and 13.58, respectively. These above six antibiotics also showed high risk in STP B, with an average RQ value of 3.26, 1.07, 8.15, 36.30, 22.32 and 26.68, respectively. The average RQ value of ceftriaxone, meropenem and trimethoprim in STP A was below 1, while they showed high risk (average RQ = 3.38, 2.23 and 1.33, respectively) in STP B. For amoxicillin, ampicillin, piperacillin, ceftriaxone, meropenem, and trimethoprim, either secondary treatment enhancement strategy through advanced biological treatment or further treatment for secondary treatment strategy can help to fulfill the discharge target for these compounds; for clarithromycin, azithromycin and levofloxacin, the combinations of consumption reduction, secondary treatment enhancement and further treatment of secondary effluent should be considered.

7.2 Recommendations for future research

1. Since the predicted removal of antibiotics in STPs highly depends on the estimation of biodegradation rate constant of antibiotics in the activated sludge, the long-time batch experiment (>72 h) on the study of biodegradation rate constant is needed to improve the accuracy of estimation.
2. Even some antibiotics (e.g., β-lactam antibiotics) can be highly removed in STPs, however, the transformation by-products of these antibiotics, which might show ecotoxicity to microorganisms or select for the resistant bacteria, were not

considered in this study. Therefore, it is necessary to further identify and quantify the transformation by-product of these antibiotics and test or estimate their ecotoxicity or MSCs.

3. During the risk assessment, the single antibiotic data on the single species were applied, to make the proposed discharge limits closer to our protection goals, further studies are required to test the ecotoxicity or MSCs of mixtures of antibiotics on the natural microbial communities.

Acknowledgement

I would like to take this opportunity to express my deep gratitude to Professor Hiroaki Tanaka, the former professor of Research Center for Environmental Quality Management, Department of Environmental Engineering, Kyoto University. As my supervisor, he supported me a lot on both my research work and daily life in Japan. I am appreciated it so much. I am also grateful to Professor Fujiwara, as my supervisor after the retirement of Professor Tanaka, he gave me many useful comments and suggestions for my dissertation and supported my further graduation procedures. I also would like to express my grateful to the Japanese Government Ministry of Education, Culture, Sports, Science, and Technology (MEXT) Scholarship unit and Kyoto University for awarding me to my Ph.D. study in Kyoto University.

During my study in Kyoto University, I have to say that it is really tough since my physical and mental conditions were both not so well. Huge support came from senseis in my lab made me feel warm and continue to finish my research work. In the beginning of my research, Lecturer Nakada guided me to build the detecting method of antibiotics by LC-MS/MS, I learned not only technology from him, but also the scientific thinking for research, which influenced me a lot in the further study. Assistant professor Ihara kindly helped me to take samples from the sewage treatment plant and communicate with the staff to obtain the operating data, which contributed a lot in my research work. And every time when I asked help from Associated Professor Nishimura and Assistant Professor Takeuchi, they always showed me the warm face and gave me kindly help. Thanks to Associated Professor Nishimura to give me comments for draft revision and submission.

The staff in sewage treatment plants supported to take samples, provide space to do batch experiments and operating data of sewage treatment plant. Thanks to their support to proceed my research.

I am also thankful to Ms. Hattori, who supported me a lot as the research program secretary. I also would like to thank all of my labmates and members in our research center for their kindly support in my study and life in Kyoto University.

Finally, I want to thank my parents and friends to give me huge support mentally.

They really gave me a lot of love when I faced obstacles, and their love supported me energy to continue and finish my research work.