Title
Removal of Animal Antibiotics for Potable Water Reclamation: A Review

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Publication Date
2015

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA

Los Angeles

Removal of Animal Antibiotics for Potable Water Reclamation: A Review

A thesis submitted in partial satisfaction

of the requirements for the degree Master of Science

in Civil Engineering

by

Rita Chang

2015

ABSTRACT OF THE THESIS

Removal of Animal Antibiotics for Potable Water Reclamation: A Review

by

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Master of Science in Civil Engineering

University of California, Los Angeles, 2015

Professor Michael K. Stenstrom, Chair

Important classes of antibiotics that are used to treat bacterial infections in humans are also being used in food-producing animals. The overuse of antibiotics for animal food production is becoming an issue of growing concern as it promotes antibacterial resistance, compromising their efficacy and effectiveness. Low concentrations of antibiotics from feedlot runoff and wastewater discharges have been reported in surface waters and groundwaters used as drinking water sources. The presence of antibiotics in drinking water sources has implications for potable reuse. Since direct potable reuse is receiving significant interest in water stressed or arid regions, many studies are now focusing on understanding the fate and removal of emerging contaminants, including antibiotics, from wastewater treatment plant effluents. This thesis provides a review of conventional and advanced treatment for the removal of antibiotics in wastewater treatment plants.

Keywords: animal antibiotics, wastewater treatment, removal, potable reuse, runoff, wastewater discharge

The thesis of Rita Chang is approved.

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2015

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1. Introduction

There are currently over 200 contaminants of emerging concerns detected in water and wastewater (EPA, 2010). The frequency of detection of emerging contaminants in waterbodies is increasing with improved analytical methods and the synthesis of new chemical compounds. Many of these chemicals are persistent and so complete degradation rarely occurs. Even for emerging contaminants that are susceptible to degradation, incessant usage and release of these chemicals continues to pollute the environment irrespectively of natural degradation (Rodriguez et al., 2013). One specific class of emerging contaminant, antibiotics, can pass through wastewater treatment plants and accumulate in aquatic environments (Kim et al., 2005; Gadipelly et al., 2014; Gulkowska et al., 2008).

Antibiotics are used to kill or inhibit the growth of bacteria. Although antibiotics are used in humans and animals, roughly 80% of their total usage is on livestock and poultry for human consumption (NRDC, 2015). Antibiotics are routinely added to the food and water of livestock to promote growth and improve feed-use efficiency. In addition, antibiotics are injected into animals when they are sick or at high risk of getting sick. The widespread use of antibiotics in animal food production has been linked to antibiotic-resistant bacterial strains (Marshall and Levy, 2011; CDC, 2014). Despite the risk associated with overuse of antibiotics, the US Food and Drug Administration (FDA) has found a 16% increase in total quantity of medically important antibiotics sold or distributed for use in food-producing animals from 2009 to 2012—with more than 32 million pounds sold in 2012. However, antibiotic sales in the US are expected to decrease with the new veterinary feed directive regulation in place to control their use (FDA, 2015). Although animal antibiotics can now only be used under the supervision of a licensed veterinarian in the US, countries such as China still use large volumes of antibiotics in food-producing animals (Collignon and Voss, 2015). About half of the 210,000 tonnes of antibiotics produced in China are administered to food-producing animals (Hvistendahl, 2012).

The continuous use of antibiotics in the meat industry has implications for water quality as they can pollute surface water and groundwater through runoff and direct infiltration, respectively. Animal antibiotics have also been identified in the effluent of wastewater treatment facilities. Given that these sources are important for indirect and direct potable reuse, reviewing treatment applications to remove antibiotics is not only a public health concern as their presence can potentially cause microbial resistance, but also a perception problem. This thesis reviews the fate of several animal antibiotics in wastewater treatment plants, along with alternative processes that can be employed to efficiently remove them for potable purposes. The optimization of conventional treatment methods for their removal in wastewater is also discussed.

2. Methodology for selecting antibiotics

Wastewater influents contain several types of antibiotics; however, due to the limited availability of information, only a few antibiotics will be selected for review. The criteria for selecting antibiotic classes were defined by considering (1) the relevance of antibiotic class to human medicine; (2) usage amongst the different animal species; and (3) their presence in wastewater treatment plants.

Based on the selection criteria mentioned above, the following antibiotic classes were selected for review:

Tetracyclines

Sulfonamides

Macrolides

Fluoroquinolones

2.1 Relevance of selected antibiotic classes to human medicine

Several classes of antibiotics used for animal feed productions are also important for human medicines. Based on the World Health Organization (WHO), macrolides and fluoroquinolones are defined as critically important antibiotics while tetracyclines and sulfonamides are classified as highly important antibiotics. Tetracyclines are occasionally considered as critically important antibiotics, depending on the country. Critically important antibiotics are the primary therapy or one of several alternatives to treat serious human diseases. Serious diseases are defined by WHO as illnesses that are likely to result in irreversible morbidity or mortality if untreated. Critically important antibiotics are also used to treat diseases caused by microorganisms that are transmitted from non-human sources or may have acquired resistance from non-human sources while highly important antibiotics satisfies only one of the above criteria (WHO, 2011). Table 1 provides the basic structure of the selected antibiotics along with comments provided by WHO that describes the importance of each given antibacterial. Additionally, a brief summary of each of their more common applications in human medicine, beyond those provided by WHO, is described below.

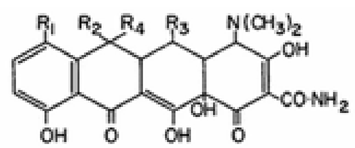
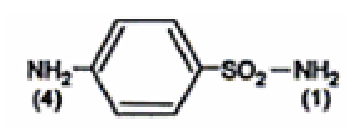
Tetracyclines: Tetracyclines are protein synthesis inhibitors that prevent the attachment of aminoacyl-tRNA to the ribosomal acceptor site. They target a range of gram-positive and gram-negative bacteria. Tetracyclines are commonly prescribed to treat skin infections, respiratory tract infections, urinary infections, infections that cause stomach ulcers, and Lyme disease (US National Library of Medicine, 2015).

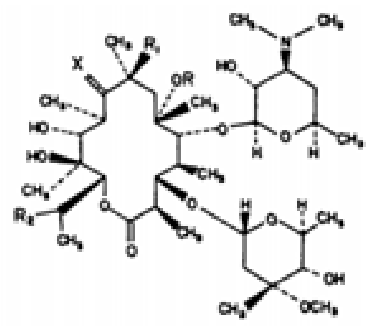
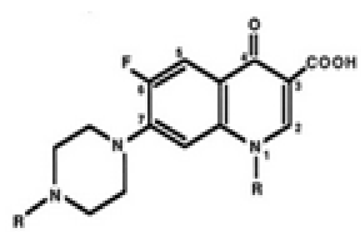
Sulfonamides: Sulfonamide antibiotics have multiple uses in human medicine. They are utilized in humans to treat pneumonia, urinary tract infections, ear infections, bronchitis, bacterial meningitis, and intestinal infections (US National Library of Medicine, 2015).

Macrolides: Macrolides have been used by humans to treat: pneumonia, diarrhea, bronchitis, skin and soft tissue infections, sexually transmitted diseases, and infections of the ears, sinuses, skin, throat, and reproductive organs (US National Library of Medicine, 2015).

Fluoroquinolones: Fluoroquinolones are a family of widely used antibiotics. Based on a study conducted by Princeton University, there was a 64% increase in global fluoroquinolone consumption between 2000 and 2010 (Van Boeckel et al., 2014). Fluoroquinolones are used to treat meningitis, bronchitis, pneumonia, anthrax, urinary tract infections, abdominal, reproductive organs in humans and infections of the skin (US National Library of Medicine, 2015).

Table 1. Structure and importance of the four antibiotics for human medicine (WHO, 2011)

Tetracycline	 (Kaji and Ryoji, 1979)	One of several antibiotics for treatment of infections due to *Brucella*, *Chlamydia* spp., and *Rickettsia* spp. *Brucella* transmission may result from non-human sources
Sulfonamide	 (Satoskar et al., 2009)	May be one of the limited antibiotic treatments available for acute bacterial meningitis, systemic non-typhoidal salmonella, and other infections *Enterobacteriaceae* may result from non-human sources

Macrolide	 *This is the chemical structure of 14-membered marcolides, which are commonly found in wastewater treatment plants (Mazzei et al., 1993)	One of several antibiotics to treat infections caused by *Legionella*, *Campylobacter*, and multi-drug resistant *Salmonella* Campylobacter and multi-drug resistant *Salmonella* may result from non-human sources
Fluoroquinolone	 (Gasser, 1992)	One of the several antibiotics to treat *Campylobacter* and multi-drug resistant *Shigella* Campylobacter and multi-drug resistant *Enterobacteriaceae* may result from non-human sources

2.2 Usage amongst animal species

Aside from the significance of animal antibiotics to humans, the usage of a particular class of antibiotics is another factor considered. Variable classes of antibiotics are used among different livestock species. For example, monensin is used exclusively for growth enhancement in cattle (Colorado State University, 2004). This difference will affect the type of animal antibiotics found in the environment. Thus, in order to quantify the general distribution of antibiotics usage, identical or closely related antibiotics used on selected livestock species have been chosen for analysis.

The most common types of animal feedlots are for cattle, swine, and poultry. The antibiotics selected for review are used in the three livestock species. The exact amounts of antibiotics administered for use in animal husbandry are currently not available. However, several studies have reported the estimated percentages for antibiotic usage in food animals. Macrolides have been administered to 42% of calves in feedlots to prevent liver abscesses, and roughly 88% of growing swine in the U.S. receive antibiotics such as tetracycline in their feed for disease prevention and growth promotion (Landers et al., 2012). Tetracyclines account for the majority of antibiotics used in food-producing animals, which is about 42% of the antibiotics consumed (FDA, 2014).

2.3 Occurrence of antibiotics in wastewater treatment plants

Global usage of antibiotics varies drastically. The distribution and classes of antibiotics found in wastewater treatment plants is country-specific. Several countries in the European Union (e.g. Sweden, Denmark, and the United Kingdom) have banned or implemented policies to reduce the use of antibiotics in animal food production while other countries depend on it heavily. Variations lie not only in the amount used, but also the types of antibiotics used.

Although antibiotics found in wastewater treatment plants are mostly a result of human use, understanding the removal efficiency of these antibiotics in treatment plants have implications on animal antibiotics as there is an overlap between human and animal medicine. Table 2 lists the occurrences of the targeted antibiotics in wastewater treatment plants from different countries—this table is not meant to be comprehensive and exhaustive. Animal antibiotics can occasionally enter wastewater treatment plants through feedlot runoff. This mechanism is especially important in regions with combined sewer systems where rainwater, domestic sewage, and industrial wastewater enter in the same collection system and are transported to a wastewater treatment plant. Antibiotics that were not removed in the treatment process will be discharged with the effluent into receiving waterbodies, affecting receiving waters and potentially impacting potable water reuse and drinking water quality.

Additionally, animal antibiotics can enter wastewater treatment plants from pharmaceutical manufacturing industries. The US Geological Survey (2014) conducted a study in 2004-2009 to determine the release of pharmaceuticals into the environment by drug manufacturers. Wastewater effluents from treatment plants that receive discharge from drug manufacturing facilities had higher pharmaceutical concentrations compared to those that did not receive discharge. Therefore, wastewater treatment plants that receive influent from pharmaceutical manufacturing industries can have elevated amounts of drug residues in their final effluents. While the USGS did not measure antibiotic concentrations, their results propose a pathway for antibiotics into treatment plants.

Table 2. Antibiotics occurrences in wastewater treatment plants

Type of antibiotics	Classes of medications	Location	Reference
Sulfonamides	Sulfamethazine Sulfamethoxazole	Wisconsin, USA	Karthikeyana and Meyerb (2006)
Tetracyclines	Tetracycline		
Fluoroquinolones	Ciprofloxacin		
Macrolide	Erythromycin-H2O		
Macrolides	Clarithromycin Roxithromycin Erythromycin-H2O	Switzerland	Mcardell et al. (2003)
Sulfonamides	Sulfacetamide Sulfadiazine Sulfamethazine Sulfamethoxazole Sulfapyridine Sulfisoxazole	Canada	Miao et al. (2004)
Tetracyclines	Doxycycline Tetracycline		
Fluoroquinolones	Ciprofloxacin Norfloxacin Ofloxacin		
Macrolides	Clarithromycin Erythromycin-H2O Roxithromycin		
Sulfonamides	Sulfamethoxazole Sulfadiazine Sulfamethazine	Spain	Gros et al. (2010)
Tetracyclines	Tetracycline Oxytetracycline		
Fluoroquinolones	Ofloxacin Ciprofloxacin Enrofloxacin Norfloxacin		
Macrolides	Erythromycin Azithromycin Roxithromycin Clarithromycin Tylosin A Spiramycin		
Sulfonamides	Sulfadiazine Sulfamethazine Sulfamethoxazole Sulfamonomethoxine Sulfapyridine	China	Zhou et al. (2013)
Tetracyclines	Chlortetracycline Doxycycline Methacycline Oxytetracycline Tetracycline		
Fluoroquinolones	Ciprofloxacin Fleroxacin Lomefloxacin Norfloxacin Ofloxacin		
Macrolides	Clarithromycin Erythromycin-H2O Roxithromycin		

2.4 Literature review selection

Scientific literature on animal antibiotics is limited; therefore, the studies reviewed expand beyond animal use. Ultimately, this thesis will provide an overview on the removal process and efficiency of conventional and advanced treatment methods for the removal of four classes of antibiotics used for human and commercial farming purposes.

3. Conventional treatment for antibiotic removal

3.1 Primary Treatment

Primary treatment of wastewater usually involves only sedimentation after screening and grit removal; it does not provide significant removal of soluble antibiotics from wastewater. Removals of antibiotics in primary treatment occur predominantly through sorption to suspended organic matter in the incoming influent (Gobel et al., 2007 and Oulton et al., 2010). Primary treatment conventionally removes approximately 35 to 40% of the five-day biochemical oxygen demand (BOD_5) and 60% of the total suspended solids (TSS). Advanced primary treatment, using chemical coagulants such as ferric chloride, aluminum sulfate, and cationic organic polymers can increase removal efficiency to as high as 60% BOD_5 and 80% TSS (Tchobanoglous et al., 2014). Unless the pharmaceuticals are strongly associated with the TSS, little removal occurs in primary treatment; and for this reason, it will not be reviewed further.

3.2 Secondary Treatment

Unlike primary treatment, secondary treatment is an important route for the removal of antibiotics. Removal of antibiotics can occur through biodegradation, sorption, volatilization, and hydrolysis. Most wastewater treatment plants use some variant of the conventional activated sludge (CAS) process for secondary treatment. Based on several studies, the two main removal mechanisms for antibiotics in the activated sludge process are biodegradation and adsorption

(García-Galán et al., 2012). Studies conducted by Li and Zhang (2010) identified adsorption as the predominant elimination route for 7 of 11antibiotics. Although biodegradation has been reported as the major removal route for 3 of the 11 antibiotics, complete mineralization of antibiotics was not reported. Microbially induced degradation can form products that are as active and/or toxic as their original parent compound. In general, studies do not report the products of degradation; therefore, readers must be conscious that degradation does not necessarily result in residuals that are less toxic and persistent.

The removal efficiency of antibiotics in wastewater treatment plants depend on process conditions, such as solid retention time, hydraulic retention time, temperature as well as antibiotic properties. Differences among chemical structures of antibiotics will effect whether it will be volatilized, degraded, adsorbed to solids or persist in treated effluent. In this section, the removal processes and efficiencies of the targeted antibiotics in biological treatment are explored.

3.2.1 Modified hydraulic and solid retention times of conventional activated sludge systems

As mentioned, process operating conditions can have a significant effect on the removal of contaminants in the treatment process. Solid retention time (SRT) and hydraulic retention time (HRT) are reportedly known to influence the treatment performance of the activated sludge process. Several studies have focused on the effects of controlling solid and hydraulic retention times for the removal of nitrogen, organic compounds, and trace contaminants (Gerrity et al., 2013, Leu et al., 2012, Soliman et al., 2007, Zeng et al., 2013, Zhu et al., 2007). However, the removal of antibiotics under different process conditions has not been widely studied. Nonetheless, one study conducted by Kim et al. (2005) evaluated the effects of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. Activated sludge primary clarifier effluents were taken from the Amherst, NY wastewater treatment plant and treated in laboratory reactions with three different process phase conditions. Phase 1 had a HRT of 24 hr and an SRT of 10 days, phase 2 had a HRT of 7.4 hr and SRT of 10 days, and phase 3 had a HRT of 7.4 hr and SRT of 3 days. The effects of different operating conditions on tetracycline removal were compared. The results showed that removal efficiency of tetracycline in phase 3 (78.4 ± 7.1%) was lower than that of phases 1 and 2 (85.1 ± 5.4% and 86.4 ± 8.7%, respectively). The removal of tetracycline in wastewater treatment process is more dependent on SRT as seen in the reduction of tetracycline removal with lower SRT while maintaining constant HRT. The removal mechanism for tetracycline was primarily dependent on sorption rather than biodegradation.

Although tetracyclines have been reported as non-biodegraded compounds, several investigators have reported varying removal rates for tetracyclines; however, none documented mineralization (Cetecioglu et al., 2014 and Prado et al., 2009). Cetecioglu et al. (2013) demonstrated that biodegradation can be responsible for the removal of tetracyclines under certain conditions. Their study showed that tetracycline could be fully or partially biodegraded along with organic substrates under anaerobic conditions.

3.2.2 Powdered activated carbon addition to activated sludge for an increase in sorption surfaces

Sorption, as mentioned, is an important route for the removal of antibiotics in wastewater treatment plants. The removal of fluoroquinolones by the sorption mechanism in secondary treatment has been reported to be around 90% (Golet et al., 2003). Li and Zhang (2010) demonstrated that three types of fluoroquinolone can be effectively removed by adsorption. The removal efficiencies for norfloxacin, ofloxacin, and ciprofloxacin in a 48-hour batch test were 91.6%, 84.4%, and 90.8%, respectively. Similar results were achieved by Giger et al. (2003) in a mass balance study, which demonstrated an 88% to 92% reduction in fluoroquinolones as a result of sorption on primary sludge and waste activated sludge. Based on this result, combined sludges are a major reservoir for antibiotics removed through the sorption mechanism.

Activated carbons are one of the most widely used adsorbents for the removal of organic pollutants due to their large surface area, high adsorption capacity, and high surface reactivity (Ranade and Bhandari, 2014). Two forms of activated carbon are frequently used in water and wastewater treatment, granular activated carbon (GAC) and powdered activated carbon (PAC). Of the two types, PAC has a faster adsorption rate and a larger adsorption capacity, which is attributed to its higher surface area, pore volume, and porosity. In addition, powdered activated carbon can be directly added to the aeration tank of the activated sludge process, commonly known as the PAC process. Previous studies have demonstrated the success of PAC application for the adsorption of microcystin toxins, phenol, and other organics (Ma et al., 2013 and Ho et al., 2011). Choi et al. (2008) assessed the removal of tetracyclines and sulfonamides from deionized and dissolved organic carbon (DOC) water by powdered activated carbon. Their research showed that tetracyclines were more easily removed compared to sulfonamides. They speculated that the increase in adsorption may be a result of the phenolic component of tetracyclines and its ability to form complexes with metal and metal oxides on the surface of the activated carbon. The main property affecting adsorption of antibiotics to activated carbon is the ionic state. The study reported that more hydrophobic sulfonamides were more easily removed; thus, removal is strongly dependent on antibiotic type. In addition, results from the DOC water suggested that dissolved organic materials (DOM) competed with the adsorption of sulfonamides and tetracyclines. Because DOM is present in waters receiving secondary treatment, implementation of PAC in conventional systems needs to be considered to ensure that removal efficiency remains high.

Yao et al. (2013) investigated the adsorption of a specific type of fluoroquinolone, gatifloxacin using sludge-derived biochar from different sources. Waste activated sludge samples were collected from several municipal and industrial treatment plants to better understand the influence of wastewater types, sludge stabilization, dehydration, and sludge dry process on sludge characteristics. The study demonstrated that most of the sludge except the sludge collected from the dry bed with lime addition was efficient in the adsorption of gatifloxacin. Since biochar are generally considered to be less efficient adsorbents compared to activated carbons, the sorption capability of fluoroquinolones to biochar confirms the use of powdered activated carbon in the activated sludge process or granular activated carbon as tertiary treatment to improve effluent quality.

3.2.3 Membrane bio-reactor as an alternative to activated sludge

Membrane bio-reactors (MBRs) are being implemented in several wastewater treatment plants in the United States as an alternative to the conventional activated sludge system. The Brightwater treatment plant in King's County, Washington is one example of a WWTP that uses a MBR. A membrane bio-reactor is a combination of biological treatment and membrane filtration. It results in higher mixed liquor suspended solids, which can translate into longer solid retention times and more active mixed liquors for better degradation of persistent contaminants. Literature suggest that compared to CAS, MBRs, although more costly, produce higher effluent quality and are more effective at removing hard-to-degrade contaminants. García-Galán et al. (2012) studied the removal efficiency of conventional activated sludge and a membrane bioreactor for sulfonamide removal from domestic (80%) and industrial (20%) wastewater influents. The source of industrial influent comes mainly from pharmaceutical and textile industries. Hollow-fiber ultrafiltration membranes (Koch) and flat-sheet microfiltration membrane modules (Kubota) MBRs were operated in parallel with the CAS treatment at the Terrassa, Barcelona, WWTP. The HRT and SRT for the treatment processes were 11.5 hrs and 10 days for CAS, 7.2 hrs and 30-40 days for Koch, and 10-20 hrs and 65-75 days for Kubota, respectively. The results from the different treatment processes were compared to determine their effectiveness for the removal of nine sulfonamides and one of their acetylated metabolites. Their study observed a maximum removal of 100% for sulfadiazine, sulfadimethoxine, sulfamethoxypyridazine, and the N^4-acetylsulfamethazine in MBR treatment. In general, the removal efficiency of MBR was higher than those obtained by CAS. Mineralization assays were not conducted to determine whether biodegradation resulted in mineralization of sulfonamide antibiotics.

The observed removal of antibiotics in MBR treatment was also studied by Schröder et al. (2012). Operational parameters and membrane selection varied from those used by García-Galán et al. (2012). Hollow-fibre ultrafiltration membranes were utilized with SRT and HRT of 15 days and 9 hrs for MBR-15 and 30 days and 13 hrs for MBR-30, respectively. The macrolide antibiotic, roxithromycin, was 57% removed by MBR-15 and 81% removal by MBR-30. Sulfamethoxazole was 55% removed at an SRT of 15 days and 64% removed at an SRT of 30 days. The sulfamethoxazole results are comparable with those obtained by García-Galán et al. (2012), who obtained 51.8% for an SRT of 30-40 days and 54.6% for an SRT of 65-75 days.

3.2.4 Glutathione S-transferase enzyme addition

Glutathione S-transferases (GST) are a major group of enzymes that play an important role in the binding and detoxification of endogenous and exogenous electrophilic compounds in prokaryotic and eukaryotic species. Detoxification of these electrophilic compounds of both endobiotic and xenobiotic origin is known to occur by conjugation reactions with glutathione. Literature have shown an association of GST enzymes in the degradation and detoxification of toxic pollutants, including morpholine, polychlorinated biphenyls, and pesticides (Kostaropoulos et al., 2001 and Emtiazi et al., 2009)

Park and Choung (2007) studied the transformation of antibiotics using GST enzymes. Two batch tests were conducted to determine antibiotic transformations. The first test did not include the addition of reduced glutathione (GSH). GSTs enzyme from rat liver, 100 mg/L of tetracycline, 100 mg/L of sulfathiazole, and 50 mg/L of ampicillin with buffer solution were used to measure transformation rates. The effects of GSTs enzymes on antibiotic transformation were compared to controls without GSTs addition. This study observed a 30% decrease in tetracycline and a 60-70% reduction in the concentration of sulfothiazole and ampicillin with GST in the presence of GSH. The transformation by GST without GSH was reduced to 23.3% for tetracycline and 45-55% for sulfathiazole and ampicillin. Based on these results, the rate of degradation by GST was mainly dependent on the type of antibiotic with tetracycline being the most difficult to transform.

Park (2012) later studied the effects of microorganisms containing glutathione S-transferases on the reduction of antibiotics. The study demonstrated that *Bifidobacterium thermophilum* and *Staphylococcus epidermidis* immobilized on alginate beads can successfully reduce tetracycline and sulphathiazole. *Staphylococcus epidermidis* removed about 70% of tetracycline. The study also noted that immobilized microorganisms produce greater amounts of enzymes compared to stationary liquid cultures.

Table 3. Advantages and disadvantages of some alterations to secondary conventional treatment.

Alteration of secondary treatment methods	Description	Advantages	Disadvantages
Control of SRT	Increase in SRT above normal operation conditions	Retention of slower-growing microorganisms for better degradation of persistent pollutants. Applicable to existing treatment plants.	Potential to promote antibiotic resistance
Membrane Bioreactor	Integration of biodegradation and membrane filtration	Longer SRT and more active mixed liquor Better effluent quality Lower HRT and less volume requirement	Low sludge settling rate Membrane fouling Generally higher cost for the same volume treated.
Powdered activated carbon treatment	Dosage of powdered activated carbon to activated sludge treatment	Improve adsorption efficiency Enhance biodegradation Removal of a wide range of organic contaminants	Large volumes of adsorbent waste and regeneration costs Potential adsorption of other contaminants before removal of antibiotics
Glutathione S-transferase	Selection for microorganisms with glutathione S-transferases	Enhance degradation	May be inadequate and ineffective w/out bioaugmentation Never demonstrated at full scale

3.3 Disinfection

Secondary treatment of wastewater is followed by disinfection for the control of waterborne microorganisms. Chlorine is the most widely used disinfectant for wastewater and water treatment. Disinfection of water by chlorine has been demonstrated to not only inactivate and destroy pathogenic organisms, but to remove pharmaceuticals. WHO reported that conventional drinking-treatment processes with free chlorine can remove about 50% of pharmaceuticals, but provides no information on the dosage and contact time (WHO, 2011). An experimental study conducted by Chamberlain and Adams (2006) confirmed the efficacy of free chlorine on antibiotic removal. Their study demonstrated that sulfonamides were readily removed by free chlorine under neutral pH while carbadox exhibited nearly complete removal. Wang et al. (2011) also reported rapid transformation of tetracyclines by free chlorine under water and wastewater treatment conditions. However, despite the success reported by previous studies, recent preliminary studies demonstrated that chlorine use in wastewater treatment may encourage the formation of new, unknown compounds with antibiotic properties.

3.3.1 Ultraviolet radiation

Natural sunlight induced photochemical degradation is one of the removal processes of fluoroquinolone. Studies carried out by Sturini et al. (2012) showed that transformation of ciprofloxacin, danofloxacin, levofloxacin, and moxifloxacin in surface water spiked at ppb concentrations (20-50 μg/L) occurred on exposure to sunlight. The study showed that ciprofloxacin and danofloxacin decomposed in about 20 minutes, while ciprofloxacin and danofloxacin degraded after 4 and 7 hours, respectively. The degradation of fluoroquinolones resulted in the formation of various intermediates that also possess antibacterial activity. Despite being effective in removing the parent compound, photochemical degradation and ultraviolet treatment may not be the best option for fluoroquinolone removal since photoproducts still possess residual antibacterial activity.

4. Advanced treatment for antibiotic removal

Conventional treatment methods are generally insufficient in the removal of contaminants from wastewaters for direct and indirect reuse purposes. Several studies have reported incomplete removal of antibiotics from conventional treatment plants. Watkinson et al. (2007) reported that sulfonamides are only partially removed in conventional treatment plants with average removals of 25%. Partial removal and incomplete mineralization of antibiotics from WWTP can have implications on receiving waterbodies since antibiotics poses human health risks and metabolites from incomplete degradation can be converted back into their original parent compound (McEvoy, 2004). Thus, wastewater treatment plants may require augmentation with advanced treatment systems. Advanced wastewater treatment combines membrane technologies, advanced oxidation processes, granular activated carbon, and other technologies to produce higher quality effluent for reclamation purposes.

4.1 Ozone

Since ozone is a strong oxidant and virucide, it can be used to degrade pollutants or transform pollutants into simpler less toxic substances. Ozone is being used in water and wastewater treatment plants to inactivate pathogenic organisms. The use of ozone to degrade pharmaceuticals and personal care products and other micropollutants in treatment plants is gaining considerable attention. In this section, the ability of ozone to remove clarithromycin, a semi-synthetic macrolide, is discussed. The degradation process and antimicrobial activity of clarithromycin under ozonation is reviewed and evaluated.

The tertiary dimethylamino group on macrolides is the site of ozone attack in Fig 1 (Lange et al., 2006). The ammonium zwitterion can undergo decay by losing the dioxygen yielding the N-oxide or dissociating into ozonide radical anion and amine radical cation. Because ozonide radical anion is stable only at high pH, it is rapidly protonated by water near pH 7 into OH and O_2. The amine radical cation deprotonates at the α-carbon and adds O_2, forming a peroxyl radical. The peroxyl radical eliminates the superoxide and the iminium ion hydrolyzes to secondary amine and aldehyde. This same pathway was observed for the ozonolysis of clarithromycin (Lange et al., 2006).

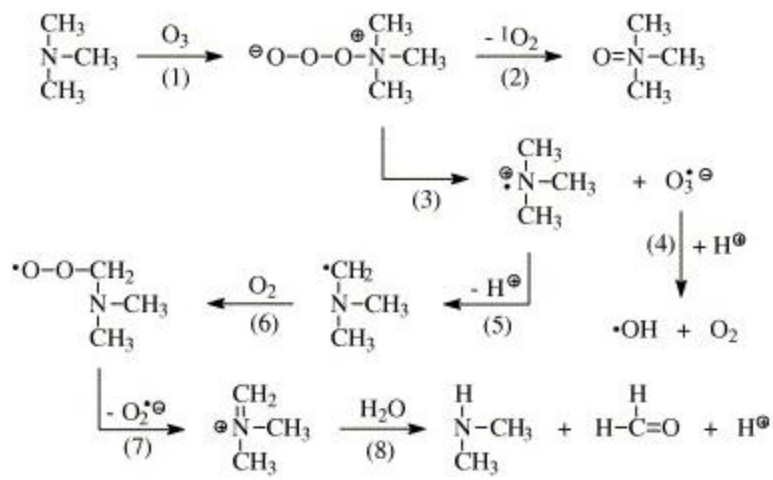


Figure 1. Pathway reactions for tertiary amines with ozone (Lange et al., 2006)

The ozonation products of clarithromycin: clarithromycin-N-oxide, demethylated clarithromycin, deaminated clarithromycin, and acetalized clarithromycin were determined to have reduced biological activity. Complete mineralization of clarithromycin can also occur with the addition of 100 times more ozone. Most of the ozonation product is an N-oxide. Because N-oxide impedes binding to rRNA, it inactivates the biological activity of clarithromycin and its function as a ribosomal antibiotic (Lange et al., 2006). These results indicate that low ozone dose is sufficient to biologically inactivate macrolides in water and wastewater treatment plants, which is essential to the control of the development of antibiotic resistant bacteria and transfer of antibiotic resistant genes. A similar study conducted by Liu et al. (2014) verifies efficiency of ozonation for the degradation of two fluoroquinolones (norfloxacin, ofloxacin) and two macrolides (roxithromycin, azithromycin). Removal efficiencies for the macrolides were greater than 99% after 10 minutes, and the same efficiency was obtained for ofloxacin and norfloxacin after 10 and 20 minutes, respectively. The ozonation rate of norfloxacin was slower compared to the other antibiotics as a result of the secondary amine group instead of the tertiary amines.

4.2 Membrane Filtration

Nanofiltration (NF) and reverse osmosis (RO) have garnered significant interest for water reuse application due to their ability to efficiently remove dissolved organic and trace organic contaminants (Steinle-Darling and Reinhard, 2008). The role of NF and RO technologies for the removal of pharmaceuticals from water has recently been demonstrated (Radjenovic et al., 2008). Studies investigating the rejection of trace contaminants by NF and RO reveal that the process is dependent on charge repulsion, size exclusion, hydrophobicity, hydrogen bonding capacity, and dipole moment (Steinle-Darling and Reinhard, 2008). Removal of hormones and antibiotics was investigated by Koyuncu et al. (2008) using nanofiltration with a molecular weight cut-off in the range of 200 - 300 Da. Their experimental results demonstrated that tetracyclines rapidly adsorbed onto membrane surfaces with nearly 70% of chlorotetracycline adsorbed after 30 min. Rejection of tetracyclines was also observed to be very high due to its high molecular weight of 450 Da or more. In contrast, sulfonamides have lower molecular weights and thus experienced less rejection. Interactions with other compounds, including natural organic matter and calcium, can also increase sulfonamide rejection (Koyuncu et al., 2008).

A study by Liu et al. (2014) using both model solutions and real secondary effluent verified the potential of nanofiltration in the removal of antibiotics from wastewater treatment plants. Their analysis for two fluoroquinolones (norfloxacin, ofloxacin) and two macrolides (roxithromycin, azithromycin) through a NFX filter with molecular weight cut-off between 150 and 300 Da under different feed solutions and operational conditions resulted in high rejections for the antibiotics.

Rejections of the four antibiotics were above 98% at the pressure of 0.2 MPa with no background organics. Steric exclusion played an important role in the rejection of antibiotics since the molecular weights were larger than the molecular weight cut-off of the membrane. Similarly, their experiment on real secondary effluent also achieved high rejection with a less obvious flux decline.

The removal of sulfonamides dissolved in distilled and river water by reverse osmosis was reported by Adams et al. (2002) using a cellulous acetate membrane. Their study achieved a rejection rate of 90% under both systems. Although most studies on nanofiltration and reverse osmosis were not carried out using secondary effluent, the results still provide information on the efficiency of membrane filtration for the removal of antibiotics. However, to better understand the behavior of antibiotics in wastewater treatment plants, studies using more complex heterogeneous environmental solutions need to be performed.

5. Implications for water reuse

With most of the Western United States suffering from drought, some states, such as California, have begun to develop projects to increase fresh water supplies. Despite being at the forefront of water recycling, California has constructed and will begin to operate its first desalination plant in Carlsbad by 2015. Desalination compared to potable water reuse will require more energy and could lead to more negative environmental impacts. Since effluent quality from many treatment facilities in California exceed federal drinking water standards, direct potable reuse is likely to be a more sustainable option. However, to guarantee the protection of human health and the environment, and to help ensure public trust, more rigorous research is needed to assess human health risk associated with trace contaminants in recycled water. Although there is no evidence of antibiotics in water supplies directly affecting human health, several studies have reported the adverse effects on aquatic life. A study by Gao et al. (2012) showed that antibiotics such as ciprofloxacin and erythromycin bioaccumulate in fish tissue. While studies have not been able to demonstrate the risk associated with antibiotics in drinking water on humans, their presence in water supplies could promote antibiotic resistance and change microbial community structure. The spread of antibiotic resistance in bacteria has important implications for global health since antibiotics are important to treat infectious diseases. If antibiotics no longer work against bacterial infections then morbidity and mortality rates in humans and animals will increase. Thus, the presence of antibiotics in water supplies is a growing public health concern.

In addition, antibiotics in wastewater treatment plants may be a disinfection by-product (DBP) precursor. Chloramination of many compounds with secondary, tertiary or quaternary amines is known to result in nitrosamine formation. Roback (2015) concluded that ten veterinary antibiotics may be an important precursor of nitrosamines such as N-Nitrosodimethylamine (NDMA) upon chloramination. Of the targeted antibiotics, several were from the tetracycline and macrolide class. Minocycline (4.9 ± 0.9) and spiramycin (3.4 ± 0.2) resulted in the highest molar conversion to NDMA at a pH of 8.4. However, under the presence of natural organic matter, NDMA formation from spiramycin was inhibited as a result of the high molecular weight. Since several water utilities and agencies in the west are now investing the time and money to understand NDMA destruction for reuse, controlling potential precursors is imperative to reducing NDMA formation and protecting human health.

6. Conclusion

Numerous treatment methods have been evaluated for the removal of antibiotics in wastewater treatment plants. Laboratory studies have shown that modified conventional and advanced treatments can remove antibiotics. However, based on their different removal efficiencies, a single treatment method has not been found for all antibiotics. Observable trends from the literatures have shown that:

(1) Tetracyclines, sulfonamides, fluoroquinolones, and macrolides are only partially removed by conventional treatment.

(2) The dominant removal mechanism for tetracyclines and fluoroquinolones in wastewater treatment plants is sorption onto activated sludge. Since sludge is applied to agricultural land, there is still potential for antibiotics removed from wastewater to enter into the environment. To enhance the removal of these antibiotics from WWTP, adsorbents can be added into the activated sludge process.

(3) Disinfection using chlorine and UV light may result in by-products that have unknown effects on public health. Ozonation as a disinfectant has demonstrated the greatest success in removing antibiotics from wastewater.

(4) It is speculated that occurrence of antibiotics in water supplies may promote the selection of antibiotic resistance genes and the development of antibiotic resistant bacteria. Antibiotic resistance has negative implications on human and animal health. The best way to minimize the presence of antibiotics in the environment and prevent the spread of resistance is to pass regulations to control and change the usage patterns.

7. Future Research

This thesis provides an overview of treatment processes for antibiotic removal to help guide future research. Although this review is not extensive and does not cover all the potential mechanisms that can be employed for antibiotic compound removal, it provides knowledge on ways to improve conventional treatment methods, and offer insight into viable alternatives. Since this dissertation did not consider cost, future research should perform a cost-benefit analysis to determine the feasibility of implementing new treatment regimes. For instance, PAC may effectively remove selected antibiotics from the liquid phase in deionized water; however, when dissolved organic material is present, the removal efficiency is reduced as a result of competition from DOM.

Laboratory experiments using mixtures of target compounds and distilled or tap water do not reflect the removal efficiencies in WWTP since water in treatment plants contains other substances that can hinder the removal efficiencies. Ozone also exhibits this problem since ozone will react with reduced material before they have a chance to react with antibiotics. Thus, future studies should focus on creating an environment reflective of wastewater treatment plants.

Much research is also needed on the removal of antibiotics from the biosolids. Because adsorption is the main removal route for certain antibiotics from liquid phase, biosolids exiting WWTP contain removed antibiotics, which can contaminant natural environments.

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