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BEHAVIOR OF ANTIBIOTICS IN THE ENVIRONMENT
พฤติกรรมของยาปฏิชีวนะในสิ่งแวดล้อม

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Abstract

Antibiotics have been used to treat human disease and to be given to livestock for disease prevention and growth promotion. Because of their large amount and widely uses, the antibiotics were found in various phases of the environment such as manure, sewage, sludge, surface water, soil, livestock and aquatic animal. The antibiotic could enter the environment and degrade in some extents depending on its physicochemical properties and the environmental conditions. Although, its existing in small amount could not cause any lethal effect but previous studies have shown the resistance effect might occur and disturb the microbial ecosystem

Keywords: antibiotics, bioaccumulation, environmental degradation, ecotoxicology

บทคัดย่อ

มีการใช้สารปฏิชีวนะในการรักษาโรคในมนุษย์และให้กับปศุสัตว์เพื่อป้องกันโรคและเสริมให้โต ด้วยเหตุที่มีสารจำนวนมากและมีการใช้อย่างกว้างขวางทำให้พบสารปฏิชีวนะในหลายตัวกลางของสิ่งแวดล้อม เช่น มูลสัตว์, น้ำโสโครก, สลัดจ์, น้ำผิวดิน, ปศุสัตว์และสัตว์น้ำ สารปฏิชีวนะสามารถเข้าสู่สิ่งแวดล้อมและมีการสลายตัวไปบ้างขึ้นกับสมบัติทางเคมีฟิสิกส์ และสภาวะของสิ่งแวดล้อม แม้สารจะมีจำนวนน้อยไม่สามารถทำให้เกิดผลถึงตายได้ แต่จากการศึกษาก่อนหน้าแสดงให้เห็นถึงความต้านทานสารอาจเกิดขึ้นได้และรบกวนระบบนิเวศของจุลชีพ

คำสำคัญ : สารปฏิชีวนะ, การสะสมทางชีวภาพ, การย่อยสลายทางสิ่งแวดล้อม,นิเวศพิษวิทยา

Introduction

Antibiotic, by definition, is a chemical substance derivable from a mold or bacterium that can kill microorganisms and cure bacterial infections[1]. People have known the antibiotic since ancient Chinese over 2,500 y ago, the same as many other ancient cultures, eg. the ancient Egyptians, Greeks and Arabs who used molds and plants to treat infections. The history of

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antibiotic was well recognized after Alexander Fleming's discovered Penicillin in 1928. With this discovery and the commercially available in 1939, Europe and the US have changed their therapeutic strategies to use antibiotics to treat human disease and to give to livestock for disease prevention and growth promotion.

The production of antibiotics begins with screening of wide ranges of microorganisms in order to find the suitable species or strains, then the selected microorganism would be tested, modified and fermented in a strong aerobic form to propagate the number for mass production. In general, antibiotics can be classified into several groups, based on their target specificity, which are aminoglycoside, ansamycins, carbacephem, carbapenems, β-lactam, cephalosporins, quinolones, sulfonamides, aminoglycosides, glycopeptides, macrolides, monobactams, polypeptides and tetracyclines. Table 1 showed some important antibiotics, class, occurrence and half life. In modern medicine, the antibiotics are widely used to cure human and animal diseases. They were used to disinfect the bacteria in fish and shrimp pond and also added to the food to promote animal growth in chicken farm. Although, antibiotics are known for their beneficial use, their resistance effect is also noticeable.

Since 1950, therapeutic usage of antibiotics in hospitals has seen to be associated with increases in multi-antibiotic resistant bacteria. It was found that the misuse of the antibiotics, for example the failure to take the antibiotic as prescription or failure to rest for sufficient recovery, could lead to the persistance of the infecteous bacterias[2]. and the antibiotic resistance effects were not shown only among the patients but also to the feed animals like chickens, pigs and cattle which were given the antibiotics in the absence of disease. Besidesh, antibiotics were found to accumulate in those farm animals[3-5]. As a result, the UK called for the restricted use of antibiotics in the 1970 and the EU has banned the use of antibiotics as growth promotional agents in 2003[6].

This regulation had affected the asian export foods. In 2002, Food Standard Agency banned the honey exported from the Republic of China because of Streptomycin and chloramphenical contamination. In 2003, the residues of nitro furans were found in shrimp exported from Vietnam and the goods were removed from European market. In order to prevent the loss of Thai export foods, Thailand had issued the regulations to restricted use of sixteen antibiotics in animal farm in 2001[7]. However, the awareness of antibiotic misuse as well as the effects on the environment is still needed.

The objectives of this paper were to briefly review the important of antibiotics to the ecosystem, their sources and the environmental conditions which affected their degradation processes.

Sources of antibiotics in the environment

Antibiotics have been used to cure human diseases since 1939 after Penicillin was commercially available. Since then many antibiotics were produced and used all over the world. The statistics of antibiotic used in each country were different. In 1996, about 10 200 tons of antibiotics were used in the European Union (EU), of which approximately 50% was applied in veterinary medicine and as growth promoters. In 1999, 13,288 tons of antibiotics were used in the EU and Switzerland, of which 65% was used in human medicine; 29% was used in the veterinary field and 6% as growth

Table 1 Some important antibiotics and their classes, occurrences, behaviors and half lives.

Classes	Antibiotics	Occurrences	Behaviors	Half lives
quinolones	ciprofloxacin norfloxacin ofloxacin oxolinic acid	hospital wastewater, municipal wastewater, treated municipal wastewater, soil sewage sludge, surface water.	high soil sorption capacity, rarely pollute water, no effect on soil organism.	<9d for oxolinic acid in water and 48 - >300 d in sediment[8].
sulfonamides	sulfonamide sulfamehoxazole sulfachloropyridazine	treated municipal wastewater, sewage sludge, surface water	great potential for leaching to groundwater, weak soil sorption capacity.	10-30 d (soil) 2.4 d (photolysis)[9].
macrolides	erythromycin clarithromycin tylosin roxithromycin	hospital wastewater, treated municipal wastewater, sewage sludge	some antibiotic (eg. roxithromycin) persists in soil in an active form, great potential to affect bacterial populations in soil to be resistant, may contaminate groundwater or surface runoff.	4-8 d for tylosin, 16 d for tiamulin, slightly degraded after 120 d for roxithromycin[10].

Table 1 (continued)

Classes	Antibiotics	Occurrences	Behaviors	Half lives
tetracyclines	chlortetracycline oxytetracycline tetracycline	surface water, hospital wastewater, municipal wastewater, sewage sludge, treated municipal wastewater, soil.	highly sorbed, immobile in soil and sediment, cation exchange is primary sorption mechanism, increasing aggregation in the presence of divalent cation	For oxytetracycline, half life is 0.26 ± 0.11 d at 43°C, 46.36 ± 4.92 d in water at pH 3.0 and 9.08 ± 4.22 d in water at pH 10.0[11]. 30 d in fresh water at pH=7 and 30 hr in sea water at pH=8[12]. 16 and 13 d in fish farm sediment[12].
nitrofurans	Nitrofurantoin nitrofurazone	hospital wastewater, soil, surface water.	Accumulated in soil, sediment and animal tissues.	For furazolidone, half life is <9 d in soil and 0.75 d in sediment[8].

promoters. In Sweden, penicillin had high use for more than 27 ton/year in 2003[13]. For USA, total antibiotic production in 2000 was approximately 16,200 tons and 70% of the production was used in livestock farming. In addition, the U.S. Food and Drug Administration Center for Veterinary Medicine (CVM) reported that about 32,540 kg antibiotics were sold in 2001 and 24,475 sold in 2002[14]. In 2006, Thailand imported >114 tons sulfonamide, >70 tons fluoroquinolones and >3 tons chloramphenical, etc[15]. These large antibiotic consumptions together with the excretion of unmetabolized antibiotics result in their findings in the parent forms everywhere.

Human and animal wastes are the important sources of antibiotic in the environment. The literature reviews showed that antibiotics could be found in sewage water, effluent from the hospital and sewage treatment plant, sludge, soil, manure, ground water and in the river. The study on antibiotics in Swedish hospital sewage water showed their concentrations were as followed: ciprofloxacin 3.6-101 μg/L, metronidazole 0.1-90.2 μg/L, sulfamehoxazole

0.4-12.8 μg/L, ofloxacin 0.2-7.6 μg/L, trimethoprim 0.6 -7.6 μg/L and doxycycline 0.6 - 6.7 μg/L[16]. Besides hospital as important point source of antibiotics, sewage is another source. The investigation of thirty-one antibiotics in the final effluents of wastewater treatment plants in five Canadian cities showed only twelve of them was found. Ciprofloxacin, clarithromycin, erythromycin, ofloxacin, sulfamethoxazole, sulfapyridine, and tetracycline were frequently detected in the effluent, however, the concentrations of these detected antibiotics did not exceed 1 μg/L [17]. Compared to the concentration of antibiotics found in Swiss sewage, the highest concentration found was norfloxacin at 0.270-0.367 μg/L[13].

Animal waste (manure) is determined to be the nonpoint source of antibiotics in the environment because of its application on land as acompost[18-19]. The range of antibiotic concentrations in manure as reported in the literature was between 250 - 2500 mg/kg depending on feeding and animal species[20]. In swine manure, the concentration of chlortetracycline and tyrosin found were as high as 7.9 mg/L and 5.2 mg/L, respectively[21]. Since most of the antibiotics are relatively soluble, the antibiotics in manure can be dissolved in water and discharged to the river. The estimated concentrations of antibiotic in untreated waste water from swine farm were between 0.004 to approximately 27 μg/L. Runoff and flush water from swine farm became another potential source of water pollution. Table 2 is an example of concentrations found in flush water of swine farm[22].

Table 2 An example of concentrations found in flush water of swine farm.

Classes	Antibiotics	Concentrations in flush water (μg/L)
sulfonamides	sulfamethazine	971
macrolides	tyrosin	116
tetracyclines	oxytetracycline	232
aminoglycoside	lincomycin	116

Fate of Antibiotics in the Environment

Many studies have shown the contamination of antibiotics in various environmental samples: water, soil sediment, plant and animal [23-25]. As most of the antibiotics are excreted unchanged in feces and urine and released into the sewer, then finally accumulated in aquatic environment. The sludge or manure could also cause land contamination when were applied to the field and the agriculture runoff or leaching would contaminate to water and groundwater later on. This is clearly seen that the antibiotics are transported from man and animal wastes to various phases of the environment and then finally return to human. However, it is noted that the fate of antibiotic in various phases of the environment is dependent upon the physicochemical properties of the antibiotic and the environmental conditions.

Physicochemical properties

Class of antibiotics is determined by its physicochemical properties. Previous study on the quantitative structure activity relationship (QSAR) showed that physicochemical properties of organic compounds such as water solubility, K_{ow} (octanol/water partition coefficient), molecular size, were related the distribution, sorption capacity and accumulation of the compounds to the environment. The study of antibiotics in aquatic environment found that sulfonamides and fluoroquinolones are the most likely water contaminants followed by macrolides because sulfonamides and fluoroquinolones had higher water solubility than macrolides[22].

K_{ow} is another physicochemical property of the compound that reflects its hydrophobicity. Previous studies have shown the linear relationships between K_{ow} and soil sorption capacity (K_d) and accumulation factor (K_B). In general, the organic compound with higher K_{ow} value had higher soil sorption capacity. However, the study on the relationship between K_{ow} and soil sorption capacity of the antibiotics had shown some differences. The study of quinolones adsorption to sediment of marine origin found that they were adsorbed to the sediment and K_{oc} (organic carbon soil sorption capacity) of efromycin in most soil were 580 to 11000[12]. A few the quinolones had high potential for bioaccumulation (log K_{ow} >2) if available as parent compounds. However, the mono and polyprotic substances are not always fitting the normal relationship between K_{ow} and bioaccumulation factor (BCF).

Furthermore, the study on the relationship between K_{ow} and soil sorption capacity of three antibiotics: oxytetracycline, sulfachloropyridazine and tylosin, also showed different results. These compounds are ionizable and the hydrophobicity of oxytetracycline < sulfachloropyridazine < tylosin. In contrast, the results on the sorption of those compounds in 11 Dutch field soils showed that the sorption capacities of sulfachloropyridazine < tylosin < oxytetracycline. This indicated that the results did not obey a relationship with log K_{ow}. In addition, the soil sorption capacities of test compounds were varied largely in each soil and were significantly related to soil pH. However, it could explain no more than 50% of the variation in the data. This study suggested that the prediction of sorption may not be able to do with only one parameter[26].

In addition, size of compound was another factor that could affect the adsorption process of antibiotic. Researchers reported that the sorption isotherm of oxytetracycline was higher than tylosin on two soil types: sand soil and sandy loam soil[20]. The soil sorption capacity of oxytetracycline was 417 and 1027 for sand soil and sandy loam soil respectively and was 8 in sand soil and 127 in sandy loam soil for tylosin. They explained that the sorption isotherm of oxytetracycline were higher than tylosin because tylosin had larger molecule so it can be adsorbed on the surface of clay particles whereas the smaller tetracycline molecule tends to penetrate into the inner clay space.

The information on the relationship of the antibiotic physicochemical properties and its behavior in the environment is not well known and need further study.

Antibiotics in the aquatic environment

The investigations of antibiotic found in different waterways are different depending upon the use in each area and the concentration of antibiotic from various sources can be different, ranged from low concentration found in ground water and river to the very high concentration found in the hospital effluent. The water quality survey of the 139 US streams reported the concentrations of various antibiotic contaminants ranged from non detectable doxycycline and enrofloxacin to 1.7 μg/L of erythromycin-H_2O[25]. In addition, the antibiotics in manure or soil could reach surface and ground water as a result of leaching and runoff process. In Germany, in the area where manure were applied to the field, erythromycin-H_2O was found in ground water at the concentration of 0.049 μg/L and detected in the effluent of wastewater treatment plant between 0.68 - 2.5 μg/L[22].

In the aquatic environment, fate and degradation of the antibiotics would be affected not only by their physicochemical properties but also by the environmental conditions such as aerobic/anaerobic conditions, water pH and temperature. The study on the antibiotic degradation of wastewater in a pilot-scale system composed of an anaerobic baffled reactor followed by a biofilm airlift suspension reactor revealed that the antibiotics: ampicillin and aureomycin with influent concentrations of 3.2 and 1.0 mg/L, respectively, could be partially degraded in the anaerobic baffled reactor: The removal efficiencies of ampicillin and aureomycin from wastewater were 16.4% and 25.9% with an hydraulic retention time (HRT) of 1.25 d, respectively. The removal efficiencies increased to 42.1% for ampicillin and 31.3% for aureomycin when HRT was 2.5 d. In contrast, the biofilm airlift suspension reactor did not display significant antibiotic removal, and the removal efficiencies of both antibiotics were less than 10% [27].

The study on the contaminated wastewater treated by conventional sewage treatment (chemical treatment and aerobic biological treatment) in Swedish sewage treatment plant found that approximately 96% of ciprofloxacin and norfloxacin were removed from waste water but only a proportion of these contaminants were removed from the sludge treated in the anaerobic digester. In Swiss sewage treatment plant, during aerobic treatment, norfloxacin was reduced from 0.270 - 0.367 μg/L to 0.048 - 0.120 μg/L while sulfamethoxazole and trimethoprim were relatively stable[28-29]. In addition, other researchers also reported that about 80-90% of fluoroquinolones ciprofloxacin and norfloxacin in wastewater treatment were eliminated but by sorption transfer to sewage sludge[30].

For the study of the antibiotic in activated sludge that entered the anaerobic digesters of municipal sewage treatment plants, it could inhibit anaerobic bacteria in the digester, especially during biogas production, resulting in a disturbance of the process[31-32]. They reported that the different behaviors among 15 antibiotics in anaerobic digestion, for example, the macrolide erythromycin was lack of inhibitory effect on biogas production while chlortetracycline (inhibit concentration at 50% level or IC_{50} 40 mg/L) and chloramphenicol (IC_{50} 15–20 mg/L) showed very powerful inhibitory effect on anaerobic digestion[31]. They explained that the effects of antibiotics on each microorganism were not the same. In their experiment, the majority of the antibiotics tested lacked activity against *acetoclastic methanogens*, being active only on the acetogenic bacteria while chloramphenicol and chlortetracycline could cause the complete inhibition of the acetoclastic methanogenic archaea. A research was also reported the inhibition effects of most antibiotics were after 7 d incubation period except metronidazol that was toxic to anaerobic bacteria with an EC_{50} (effective concentration at 50% level) of 0.7 mg/L[33].

Among various antibiotic sorption to soil and sediment, the order of antibiotics which were strongly desorbed to soil and sediment were tetracycline >quinolones >sulfonamide > macrolide >aminoglycoside and β-lactam. As a result, quinolones and sulfonamide are most likely water contaminants followed by macrolides and tetracycline are likely to be found in water and soil near their sources[22].

Antibiotics in soil and sediment

A large fraction of antibiotic administered to farmed fish, swine or chicken is released into the environment, either by leaching from uneaten medicated pellet feed, or through urinary and fecal excretions. As a result, the major route of antibiotic entering agricultural soil is through the use of contaminated manure and sludge as fertilizers. Several studies have reported the antibiotic pathway was from manure to soil and sediment. The analysis of subsurface soils which were applied with manure showed that at 60 cm depth, oxytetracycline was found with the concentration of 5 μg/kg[18, 34]. Investigations reported the finding of tetracycline at 30 cm depth soil with the concentration of 170 μg/kg. In sediment, the residues of antibiotic have been found mostly because of the use of antibiotic as feed additives in fish, chicken or swine farms[4, 34-35]. Fate and behavior of antibiotic in soil were dependent upon its physicochemical properties and soil characteristics. In general, the adsorption of antibiotic varies with soil type which determined by soil composition. Antibiotic could adsorb to soil particles, especially to clay, organic carbon and the cation.

A researcher conducted the sorption experiments on two soil types; type 1 low organic carbon and high clay content and type 2 high organic carbon and low clay content[36]. The results from all test compounds conformed to linear and Freundlich isotherm. In addition, the adsorption seemed to be positively correlated with the organic carbon content in soil and varied with test compounds. Ofloxacin was strongly adsorbed while clofibric was weakly adsorbed. The lysimeters confirmed the study that clofibric had high mobility while ofloxacin was strongly adsorbed to soil and did found in the leachate[36]. The experiment on the adsorption of oxytetracycline and tylosin to two different soil types: loamy sand and clay loam, showed that the sorption capacity of both compounds in clay loam soil were higher than those found in loamy sand[20]. It was also found that soil that contains divalent cation (eg. ca^{2+} and Mg^{2+}) could bind to antibiotic. For example, previous studies had shown that oxytetracycline, oxolinic acid and Flumequine were found to bind with calcium and magnesium ion and other substances[12]. and tetracyclines could be rapidly metabolized and form relatively stable complex with metal ion[17].

Several studies have performed the experiment on the sorption of antibiotic to soil containing oxide particles and proposed the possible mechanisms which antibiotic would sorb to organic matter phases by cation exchange process and complexation with metals. It also noted that complexation with metal was dependent on soil pH. For example, the result of tetracyclines adsorption in aqueous Ca-montmorillonite and Na-montmorillonite experiments showed that the adsorption of tetracyclines on montmorillonite at pH between 1.5 to 8.7 was through complexation with Ca^{2+} in the inter layer. While at pH = 11, Ca-tetracycline complex were also observed, but appeared to be external to the montmorillonite inner layer and the tetracyclines sorption capacity was reduced.

In 2005[37], an investigation on the sorptions of three tetracyclines (oxytetracycline, tetracycline and chlortetracycline) by several soils with different pH and cation exchange capacity (CEC) found that all three tetracyclines are highly sorbed, especially in acidic and high clay soils[37]. The sorptions of tetracyclines by soils from different aqueous solution of 0.01N $CaCl_2$ and 0.001N $CaCl_2$ showed that the higher Ca^{2+} from $CaCl_2$ solution, the higher sorption capacity. It was suggested that cation exchange is more favorable than hydrophobic partitioning process due to Ca^{2+} or other cation (metal ion) could complex with tetracycline. In conclusion, the sorption of tetracyclines was dependent on pH (range from 4 to 8) and CEC.

The results from those studies indicated that complexation between antibiotic and cations in organic matter were dominant as compared to hydrophobicity partitioning process between antibiotic and soil organic carbon. The strongly bound between antibiotic and metal ion could explain the high amount of sorption observed, as a result, the estimation of soil sorption capacity did not follow the relationship with K_{ow} values as found in other organic compounds.

Further studies in sediment, the antibiotic in manure lagoons was normally conducted because of its high organic matter and wastewater discharged from the farm. The analysis of oxytetracycline in fish farm sediment, following therapeutic use, were at the concentration of 0.1 -10 mg/kg[38]. Other also reported the concentrations of oxytetracycline at 0.1 and 4.9 mg/kg dry matter in natural sediment samples[12]. In addition, the study on the effects of environmental condition to antibiotic accumulation, oxytetracycline may reach 0 to 16 mg/kg sediment and may be very stable in fish farm sediment at low temperature and stagnant anoxic condition.

The effect of antibiotic on soil microorganism is another important issue. Since microorganisms in soil have provided important ecosystem services, such as nutrient recycling, organic matter mineralization and degradation of pollutants. In addition, microorganism also plays a major role to detoxify the pollutant in soil and sediment. Although, the antibiotic concentrations mostly found in soil and sediment were not at therapeutic levels to cause inhibitory effect on bacteria population. However, they may influence the selection of antibiotic resistant bacteria in the environment and the resistant microorganism species might prolong the persistence of some pollutants[21]. A report on a microcosm experiment in natural marine sediment showed the production of $^{14}CO_2$ from the complete mineralization of pyrene from ciprofloxacin during 11 weeks. For sediment samples, bacterial community structure was analyzed after 7 weeks[39]. The results concluded that the ciprofloxacin can be a potential threat to both bacterial diversity and an essential ecosystem service[39].

Bioaccumulation of antibiotics in plants and animals

Bioaccumulation of antibiotic could be found in both plants and animals. The experiment on the uptake of sulfonamides, tetracyclines, fluoroquinolones, macrolides and β-lactams, in lettuce and carrot showed that phenylbutazone, oxytetracycline and enrofloxacin could reduce plant growth. The uptake data for whole carrot and carrot root peelings indicated the majority of antibiotic accumulate at the outer layer of the carrot. It was also found that the uptake of antibiotic via root system had no relationship with log K_{ow}. This confirmed that the behavior of antibiotic in the environment is poorly related to hydrophobicity of antibiotic but might be determined by other factors such as cation bridging at clay surface or complexation as mentioned earlier[23].

In Thailand, the study on antibiotic accumulation mostly focused on shrimp. The analysis results of oxytetracycline residue in 5,205 frozen black tiger shrimp samples collected from Thai export products in 1994, 1995, 1996, 1997 and 1998 showed the contamination ranges of 0.16-0.49, 0.14-1.46, 0.13-3.31, 0.13-0.69 and 0.10-2.05 mg/kg, respectively. Although less than 1 % of total samples were found to be contaminated with oxytetracycline at higher than Codex Maximum Residue Level or MRL (0.20 mg/kg), the trend of detected samples during 1994-1998 does not change[3]. In addition, oxolinic acid and oxytetracycline residues in shrimp muscle purchased from Klongtoei market in Bangkok, Thailand, during August 1996 to January 1997 were determined and the results indicated that oxytetracycline residue level found in giant freshwater prawn was ranged from 0.223 to 1.407 mg/kg and was ranged from 0 to 0.225 mg/kg in Banana prawn. The oxolinic acid residue was found only in giant freshwater prawn in January 1997 with the concentration of 0.241 mg/kg in shrimp muscle[40].

Environmental Degradation and Half Life

Antibiotics may be metabolized in the animal before entering the environment. It may be absorbed, transported, bioaccumulated or undergo transformations (under biotic and abiotic conditions) or reactivation. It was found that some antibiotics were able to reactivate in the environment such as chloramphenical gluoronide was converted to the parent compound chloramphenical in liquid manure[12].

For most antibiotics, abiotic degradation (e.g. pH, temperature, light intensity, etc. of the environment) would dominate its existence in aquatic environment while microbial degradation is its main degradation pathway in sediment. The physicochemical properties of the antibiotics (class of antibiotic) and soil type also play a role in degradation process.

Degradation under environmental conditions

The environmental conditions such as pH, temperature, and light intensity are important factors which affect the degradation of antibiotic in the environment. This is shown by the experiment of oxytetracycline degradation in deionized water under variuos controlled laboratory conditions: pH, temperature and light. Experiments on antibiotic indicated they were more stable at low temperatures (4°C) in opposite to high temperature (43°C) that increased oxytetracycline degradation and resulted to a very short half-life of 0.26 $\pm$ 0.11 d [11]. Moreover, oxytetracycline exposed to light could increase degradation rates threefold higher than those found under dark conditions. In acidic conditions (pH 3.0) oxytetracycline was more stable (half-life = 46.36 $\pm$ 4.92 d) but for alkaline conditions (pH 10.0) degradation rate was increased (half-life = 9.08 $\pm$ 4.22 d). The study of oxytetracycline in water kept in the dark for 2 months also found that oxytetracycline was very stable but its concentration decreased dramatically after exposed to the light.

Researchers studied the antibiotic degradation under high temperature environment by following tetracycline degradation in chicken and pork processing[5]. They reported that both thermal processing conditions (microwave heating and boiling) reduced the initial concentration of tetracycline between 56–82% and at the same time, the treatments caused the formation of the corresponding anhydrotetracycline, a degradation product of tetracycline. Comparison between two heating process, the initial sample temperature was 17± 25 °C and could reach to 97.6 °C in boil samples and 130 °C in microwave samples. The analysis of degradation product showed that test compounds in microwave samples degraded more than those found in boil samples and the higher temperature in the microwave was responsible to those results.

Microbial degradation is an important process to detoxify antibiotic in soil. A study on degradation of five antibiotic residues in marine sediments: ampicillin, doxycycline, oxytetracycline, thiamphenicol and josamycin were significantly degraded except josamycin with wide range of detections and gram-positive bacterial groups, the *Flavobacterium–Cytophaga–Bacteroides* group, and the *proteobacteria* subdivisions were responsible for the antibiotic degradation activities[4].

In 2006, a report on the fermentative degradation of hydroquinone in nearly-homogeneous mixed methanogenic cultures obtained from freshwater sediments and sewage sludge showed Gram-negative short rods, together with hydrogen- and acetate-utilizing methanogens predominated in these cultures and degraded hydroquinone to form phenol. The forming of phenol during hydroquinone degradation indicated that reductive dehydroxylation was the primary event in degradation of this antibiotic[41].

Degradation of antibiotics in aquatic environment

Hydrolysis and photolysis might be important process for the degradation of antibiotic in water. As mentioned earlier that the behavior of antibiotic in aquatic environment was dependent upon its physicochemical property, the water quality and environmental conditions. The degradation of the antibiotic in aquatic environment was controlled by those factors as well. In 2007, the results of the experiments on oxytetracycline, oxolinic acid, flumequine and florfenicol degradation in three types of water (demonized water, freshwater and seawater) under abiotic condition found that florfenicol was not degraded by hydrolysis or photolysis phenomena[42]. In contrast, approximately 20% of oxytetracycline was hydrolyzed after 14-d exposure at temperature 8 °C and about 70% degraded under photolysis in freshwater and seawater. With the same exposure, about 10% of the oxolinic acid and flumequine in seawater were also degraded under photolysis[42]. The study of oxytetracycline in fresh water and sea water reported a half life of 30 d in fresh water (pH=7) and only 30 hr in sea water (pH=8) and seven metabolites of tetracycline after photodecomposition conditions similar to natural waters in a fish culture pond were found[12].

For the degradation of antibiotic in water, a revealed preliminary survey on the occurrence of antibiotics in the U.S., municipal wastewater that sulfonamide degradation in sewage may be negligible while aminoglycoside, β-lactam and macrolide are likely to biodegrade to a greater extent than quinolones sulfonamide and tetracycline[22].

Degradation of antibiotics in soil and sediment

In soil, the degradation of antibiotics was depended on similar factors which control the degradation process in aquatic environment which are the soil properties, abiotic conditions and aging. However, unlike photolysis of antibiotic in water, photodecomposition is a negligible process to detoxify antibiotic in soil[26]. This is shown by the investigation of antibiotic degradation under sun light showed that quinolones and tetracycline degrade slowly if the exposure to sunlight is limited[22]. The investigation of light effect to oxytetracycline, chlortetracycline sulfanilamide, sulfadimidine, sulfadimethoxine, sulfadiazine, sulfapyridine and fenbedazole showed that degradation of sulfanilamide and fenbedazole due to photolysis was negligible but for tetracycline the concentration clearly decline and followed first order kinetics.

Anoxic condition is another factor that involved with degradation process. It was found that oxytetracycline degradation in the bottom deposit of fish farm was relatively slow because of the anoxic condition. Its half life was approximately 10 weeks while and co-workers[12]. quoted the result from co workers that oxytetracycline half life in the sediment of two cages at fish farm were 16 and 13 d In addition, the investigation of oxyteracyclines degradation in soil interstitial water found three degradation products: 4-epi-oxyteracycline (EOCT), α-apo-oxyteracycline (α-apo-oxytetracycline) and β-apo-oxyteracycline (β-apo-oxytetracycline)[43]. Half life of oxytetracycline in soil interstitial water were in the order of 2 d in the form of EOCT to 270 d in the form of β-apo-oxytetracycline. They also reported that quinolones could adsorb in the sediment and were more persistent than sulfadiazine and trimetroprin. For florfenicol, it could degrade to form florfenicol amine and half life was 4.5 d[43].

Investigation on the degradation of various antibiotics in liquid manure found that oxytetracycline has considerable degraded in contrast to rapid loss of tylosin under both aerobic and methanogenic condition while β-lactam, fluoroquinolone and sulfonamide degradation were very slow. Partial biodegradation was observed with β-lactam and no biodegradation for ciprofloxacin and sulfonamide[22].

Ecotoxicology of antibiotics

Antibiotic had a direct impact on microorganism. However, the scientific paper on the effect of antibiotic to microbial ecology was not much and only small amount of antibiotics found in the environment. Although the accumulation was not high enough to cause any adverse effects to plants or animal but repeated exposure to the antibiotics from manure application or waste disposal may be able to increase the antibiotic resistant bacteria especially those resistant pathogen which could pose treats to human health coworkers[44]. As mentioned earlier that some antibiotics could inhibit the activities of microorganism in anaerobic digestion and decrease the efficiency of the system.

In aquatic environment, antibiotic was normally found in range of micrograms per liter. Although these concentrations were not lethal to aquatic invertebrates or fish which usually were killed in high milligrams per liter range. Neither the concentrations caused sublethal effect which occurred in the concentration range of low milligrams per liter[17]. However, previous studies had shown the effects of antibiotic in aquatic environment, for example, a study employed disc sensitivity test method to perform the experiment on isolated bacteria obtained from three different sources: infected black tiger shrimps, pond water from shrimp farm and Chanthaburi River[45]. The experiment found that those bacteria were sensitive to Chloramphenicol and Norfloxacin but mostly resisted to oxytetracycline. The minimum inhibitory concentration (MIC) for oxytetracycline was between 0.1-50 g/mL. Furthermore, the transferring process of tetracycline, sulpamethoxazole-trimethoprim, streptomycin and erythromycin resistance gene from *Vibrio cholerae* to *Esherichia coli* and *Esherichia coli* to *Esherichia coli* and suggested that the mobility of the antibiotic resistance gene may further contribute to the development and spread of antibiotic resistance among pathogenic bacteria[46].

In soil, antibiotics are tightly held by the clay particles but they are still biologically active and select for antibiotic resistant bacteria by providing selective environment. The investigation of the effect of antibiotics (sulfachloropyridazine, oxytetracycline and tylosin) on soil bacteria found to increase tolerance in the bacteria communities[19]. Moreover, the study on shrimps fed with either oxytetracycline at the concentrations of 5 g/kg feed or higher for 7 consecutive days showed significantly higher resistant to experimental infection by *Vibrio parahaemolyticus*[47].

Conclusion

Antibiotic has been used for many years with no trend of decreasing. It is used not only for curing human disease but also for animal husbandry, swine and poultry production. In Thailand, the record has shown a large amount of antibiotic imported but their usage in swine, chicken or shrimp farms were not well documented. In addition, the distribution of antibiotic in the environment was not studied and literature review showed that most of the studies were conducted only in accordance with export needs such as the study of antibiotic accumulation in shrimp or frozen food. As a result, antibiotic may have a chance to accumulate more and more in the environment because of too little recognition.

This paper had reviewed many previous studies and found that the pathway of antibiotic either from man or animal could cause water or land contamination. Although those concentrations which accumulated in various phases were low and did not cause lethal effects to any organism but the studies indicated that the resistance effect might occurred to microorganism in the environment. If it was so, the microbial ecosystem may be disturbed and may affect human health if the pathogen is survived. As a result, the study on the ecological effects of the antibiotic is still needed in order to protect our selves and the environment.

References

(1) WordNet Search 2009. Antibiotic. Available online at http://wordnetweb.princeton.edu/perl/webwn?s=antibiotic

(2) Spanggaard, B., Jorgensen, F.G.L. and Huss, H.H. 1993. Antibiotic resistance in Bacteria Isolated from Three Freshwater Farms and an Unpolluted Stream in Denmark. Aquacult. 115, 195-207.

(3) Sangvirun, P. and Vitchaivutivong, S. 2000. Oxytetracycline Residue in Frozen Exported Black Tiger Shrimp. Bull. Dep.Med. Sci. 42 (2), 155-159

(4) Maki, T., Hasegawa, H., Kitami, H., Fumoto, K., Muneka, Y. and Ueda, K.2006. Bacterial Degradation of Antibiotic Residues in Marine Fish Farm Sediments of Uranouchi Bay and Phylogenetic Analysis of Antibiotic-Degrading Bacteria Using 16S rDNA Sequences. Fish. Sci. 72 (4), 811-820.

(5) Gratacos-Cubarsi, M., Fernandez-Garcia, A., Picouet, P., Valero-Pamplona, A., Garcia-Regueiro, J-A and Castellari, M. 2007. Formation of Tetracycline Degradation Products in Chicken and Pig Meat under Different Thermal Processing Conditions. J. Agric. Food Chem. 55, 4610-4616.

(6) Wikipedia (2009). Antibiotic. Available online at http://en.wikipedia.org/wiki/List_of_antibiotics

(7) Ministry of Public Health, 2003. Food Standards for Some Contaminated Chemicals. Notification of the Ministry of Public Health No. 268. 2003

(8) Boxall, A.B., Fogg, L.A., Kay, P., Blackwell, P.A.,

Pemberton, E.J. and Croxford, A. 2004. Veterinary Medicines in the Environment. Rev Environ Contam. Toxicol. 1801-1891.

(9) García-Galán, M.J., Díaz-Cruz, M.S. and Barcelo, D. 2008. Identification and determination of metabolites and degradation products of sulfonamide antibiotics. Trends anal. Chem. 27(11), 1008-1022

(10) Schlusener, M. P., Bester, K. 2006. Persistence of antibiotics such as macrolides, tiamulin and salinomycin in soil. Environ. Pollut. 143 (3), 565-571

(11) Doi, A.M. and Stoskopf, M.K. 2000. The Kinetics of Oxytetracycline Degradation in Deionized Water under Varying Temperature, pH, Light, Substrate, and Organic Matter. J. Aquat. Anim. Health, 2000 (12), 246-253

(12) Dobel, S., Palludan, E. and Jansen, F. 2002. Environmental Fate and Occurrence of Veterinary Medicinal Products. Environmental Project no. 659. Danish EPA Publication Database. Available online at http://www.mst.dk/udgiv/publications/2002/87-7944-9741-9/html/

(13) Linberg, R 2006. Determination of Antibiotics in the Swedish Environment with Emphasis on Sewage Treatment Plants. Department of Chemistry, Environmental Chemistry, Umea University, Sweden. 31p

(14) MacMillan, J.R., Schnick, R. and Fornshell, G. 2002. Volume of Antibiotics Sold (2001 and 2002) in US Domestic Aquaculture Industry National Aquatic Association, WV. U.S.A. 6p. Available online at http://www.thenaa.net/downloads/AETF_antibiotic_use_white_paper

(15) Ministry of Commerce, 2005. Import of drugs, Pharmaceutical Product and Derivatives. Department of Foreign Trade, Ministry of Commerce (in Thai). Available online at http://www.dft.moc.go.th/aStructure_th.asp

(16) Linberg, R., Jarnheimer, P-A, Olsen, B., J ohansson, M. and Tysklind, M. 2004a. Determination of Antibiotic Substances in Hospital Sewage Water Using Solid Phase Extraction and Liquid Chromatography/Mass Spectrometry and Group Analogue Internal Standard. Chemosphere. 57(10), 1479-1488.

(17) Sheng, X Bishay, F., Chen, M. and Metcalfe, C. 2004. Occurrence of Antimicrobials in the Final Effluents of Wastewater Treatment Plants in Canada. Environ Sci. Technol. 38, 3533-3541.

(18) Hamscher, G., Sczesny, S., Hoper, H. and Nua, H. 2002. Determination of persistent Tetracycline Residues in Soil Fertilized with Liquid Manure by High Performance Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry. Anal. Chem. 74:1509 -1518

(19) Schmitt, H. 2003a. Antibiotics cause Pollution-Induced Community Tolerance in Soil Microbial Communities. Proceedings on the European Conference on Pharmaceuticals in the Environment 14-16 April 2003, Lyon, France.

(20) Chander , Y., Kumar, K. Goyal, S.M. and Gupta, S.C. 2005. Antibiotic Activity of Soil-Bound Antibiotics. J. Environ Qual 34, 1952 -1957.

(21) Madison, W.I. 2004. Antibiotics in the Environment. J. of Environ. Qual. 33 (1). Available online at http://www.jeq.scijournals.org/cgi/content/abstract/33/1/250

(22) Huang, C-H., Renew, J., Smeby, K., Pinkston, K. and Sedlak, D. 2005. Assessment of Potential Antibiotic Contaminants in Water and Preliminary Occurrence Analysis. Georgia Institute of Technology. Available online at http://www.vcowr.siu.edu/updates/pdfn/v120-A4.pdf

(23) Boxall, A.B , Johnson, P., Smith, E.J., Sinclair, C.J., Stutt, E and Levy, L.S.2006. Uptake of Veterinary Medicines from Soils into Plants. J. Agric. Food Chem. 54, 2288-2297

(24) Chainark, P., Nawat, K. and Samranrat, N. 2000. Monitoring of Oxytetracycline Residues in Black Tiger Shrimp Pond Soil in Phangnga Province. Technical Paper No 46/2000, Phangnga Coastal Aquaculture Development Center. 10 p.

(25) Kolpin, D.W., Furlong, E.T., Meyerm M.T. Thurman, E.M., Zaugg, S.D., Barber, L.B. and Buxton, H.T. 2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in u<S. Streams, 1999-2000: A National Reconnaissance. Environ. Sci. Technol., 36(6), 1202-1211.

(26) Schmitt, H. 2003b The Sorption of Veterinary Pharmaceuticals to 11 Different Field Soils. Proceedings on the European Conference on Pharmaceuticals in the Environment 14-16 April 2003, Lyon, France.

(27) Zhou, P., ASCE, M., Su, C., Li, B. and Qian, Y 2006. Treatment of High- Strength Pharmaceutical Wastewater and Removal of Antibiotics in Anaerobic and Aerobic Biological Treatment Processes. J. Environ. Eng. 132 (1), 129-136.

(28) Linberg, R. Jarnheimer, P-A, Olsen, B., Johansson, M and Tysklind, M. 2004b. Behavior of Fluoroquinones and Trimethoprim during Mechanical, Chemical, and Active Sludge Treatment of Sewage Water and Digestion of Sludge. Environ. Sci. Technol., 40(3), 1042-1048.

(29) Linberg, R 2006. Determination of Antibiotics in the Swedish Environment with Emphasis on Sewage Treatment Plants. Department of Chemistry, Environmental Chemistry, Umea University, Sweden. 31p

(30) Giger, W., Alder, A.C., Golet, E.M., Kohler, H-P.E., McAdrdell, C.S., Molnar, E., Siegrist, H and Suter, M.J-F. 2003. Occurrence and Fate of Antibiotics as Trace Contaminants in Wastewater, Sewage Sludges, and Surface Waters. Chimia 57, 485-491.

(31) Sanz, J.L., Rodŕiguez, N. and Amils, R. 1996. The action of antibiotics on the anaerobic digestion process. Appl. Microbiol. Biotechnol. 6 (5-6), 587-592

(32) Zhou, P., ASCE, M., Su, C., Li, B. and and Qian, Y 2006. Treatment of High- Strength Pharmaceutical

Wastewater and Removal of Antibiotics in Anaerobic and Aerobic Biological Treatment Processes. J. Environ. Eng. 132 (1), 129-136.

(33) Gartiser, S., Urich, E. Alexy, R. and Kümmerer, K. 2007. Anaerobic inhibition and biodegradation of antibiotics in ISO test schemes Chemosphere 66 (10), 1839 -1848.

(34) De Liguoro, M., Cibin,V., Capolongo, F., Halling-Sorensen and Montesissa, C. 2003. Use of Tetracycline and Tyrosin in Intensive Calf Farming: Evolution of Transfer to Manure and Soil. Chemosphere 52: 203-212.

(35) Thavornyutikarn, M. and Thongnun, J. 2000. Determination of antibiotic residues in soil and black tiger shrimp muscle from culture ponds in Chachoengsao province from 1993 to 1996. Technical Paper No. 32/2000, Chuchoengsao Coastal Aquaculture Development Center. 9 p

(36) Drillia, P. 2003. The fate of Pharmaceuticals on Soil. Proceedings on the European Conference on Pharmaceuticals in the Environment 14- 16 April 2003, Lyon, France.

(37) Sassman, S.A. and Lee, L.S. 2005. Sorption of Three Tetracyclines by Several Soils: Assessing the Role of pH and Cation Exchange. Environ. Sci. Technol, 39, 7452-7459.

(38) Tantiyaswasdikul, P. 2008. Oxytetracycline. Department of Pharmacology, Faculty of Phamaceutical Sciences, Chulanlongkorn University, Bangkok. Available online at www.fao.org/docrep/w4601e/w4601e0b.htm

(39) Näslund, J., Hedman, J.E. and Agestrand, C 2008. Effects of the antibiotic ciprofloxacin on the bacterial community structure and degradation of pyrene in marine sediment. Aquat. Toxicol. 90 (3), 223-227.

(40) Tangtrongpiros, J., Chansue, N. and Koeypudsa, W. 1998. Determination of Oxolinic acid and Oxytetracycline Residues in Giant Freshwater Prawn (*Macrobrachium rosenbergii*), Banana Prawn (*Penaeus merguiensis*) and Giant Black Tiger Prawn (*P. monodon*). In Proceedings of the 24th Annual Conference of the Thai Veterinary Medical Association (TVMA) and the 4th Conference of the Veterinary Practitioner Association of Thailand to commemorate the auspices p.133-140.

(41) Szewzyk, U., Regine Szewzyk, R., and Schink, B. 2006. Methanogenic Degradation of Hydroquinone and Catechol via Reductive Dehydroxylation to Phenol. FEMS Microbiol. Lett. 31(2), 79-

(42) Pouliquen, H., Delépée, R., Larhantec-Verdier, M., Morvan and Le Bris, H. 2007. Comparative hydrolysis and photolysis of four antibacterial agents (oxytetracycline oxolinic acid, flumequine and florfenicol) in deionised water, freshwater and seawater under abiotic conditions Aquacult, 262 (1), 23-28.

(43) Halling-Sorenson, B., Lykkeberg, A., Ingerslev, F., Blackwell, P. and Tjornelund, J. 2003. Characterisation of the Antiobiotic Degradation pathways of the Oxytetracyclines in Soil Interstitial Water Using LC-MS-MS. Chemosphere, 50, 1331-1342.

(44) Kochansky, J. 2000. Analysis of Oxytetracycline in Extender Patties. Apidologie 31, 517 -524

(45) Worasing, S. and Damruk, B. 1998. Antibiotic Sensitivity of Bacteria Isolated from Chanthaburi River, Water in Shrimp Ponds and Infected Black Tiger Shrimps, Penaeus monodon Fabricius, in Chanthaburi Province. Technical paper No.16/1998. Trat Coastal Aquaculture Station. 14 p.

(46) Zahid, H.M., Mahal, Z. and Chowdhury, M.R. 2009. Intergenic and Intragenic Conjugal Transfer of Multiple Antibiotic Resistance Determinants among Bacteria in the Aquatic Environment of Bangladesh. Afr. J. Biotechnol, 8(2), 155-160. Available online at http://www.academicjounrnals.org/AJB

(47) Tabthipwan, P., Areechon, N., and Muksig, Y. 1989. Residues and Effects on Growth and Diseases of Some Antibiotics in Penaeus monodon Fabricius. National Research Council. 106 p.