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Transformation of erythromycin during secondary effluent soil aquifer recharging: Removal contribution and degradation path

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ABSTRACT

Erythromycin (ERY), a widely used antibiotic, has recently been detected in municipal secondary effluents and poses serious threats to human health during wastewater reusing. In this study, the removal, fate, and degradation pathway of ERY in secondary effluent during soil aquifer treatment was evaluated *via* laboratory-scale SAT tests. Up to a 92.9% reduction of ERY in synthetic secondary effluent was observed in 1.0 m depth column system, which decreased to 64.7% when recharged with wastewater treatment plant secondary effluent. XRD-fractionation results demonstrated that the transphilic acid and hydrophobic acid fractions in secondary effluent compete for the adsorption sites of the packed soil and lead to a declined ERY removal. Moreover, aerobic biodegradation was the predominant role for ERY removal, contributing more than 60% reduction of ERY when recharged with synthetic secondary effluent. Destruction of 14-member macrocyclic lactone ring and breakdown of two cyclic sugars (L-cladinose and D-desosamine) were main removal pathways for ERY degradation, and produced six new intermediates.

Introduction

Antibiotics are the most widely used pharmaceutical compounds for preventing or treating human/animal diseases, however, excretion of those incompletely metabolized antibiotics into ecosystem has become a worldwide environmental issue (Chang et al., 2010; Niu et al., 2013). For example, it is estimated that about 138 g antibiotics/year were consumed per person in China (ten times higher than in America), and 60–85% of those antibiotics were directly discharged into sewage drains and the environment *via* human excrement (Tian, 2010; Niu et al., 2013). Recent studies reported that abundant antibiotics were detected in wastewater treatment plant (WWTP) effluent (Costanzo et al., 2005; Batt et al., 2006; Arye et al., 2011), with macrolides, quinolone and sulfonamide being most prevalent. For their genotoxicity to bacterial biomass even under low concentration (Hernando et al., 2006; Watkinson et al., 2009), these antibiotics are recognized as priority pollutants during wastewater treatment and secondary/tertiary effluent reuse (Onesios and Bouwer, 2012).

Of the pharmaceutical antibiotics in use, erythromycin (ERY) is one of the most frequently detected compounds in surface water, secondary effluent, and other water bodies, which is used worldwide to treat infectious diseases (Kummerer,

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2009; Serisier and Martin, 2011; Gao et al., 2012). Specifically, high ERY concentrations (0.08–2.5 μg/L) in Spain and Switzerland WWTP effluents have been previously reported (Alder et al., 2001; Suárez et al., 2010). For its continual input and persistence in natural environment (Hernando et al., 2006), ERY has been selected as a "pseudopersistent" contaminant by USEPA and "high priority level" contaminant by the Global Water Research Coalition (GWRC, 2008; USEPA, 2010). Alexy et al. (2004) reported that ERY could not be readily biodegraded in the Closed Bottle Test at initial concentration of 2.46 mg/L, and Gartiser et al. (2007) found a concentration of 167 mg/L ERY inhibited carbon removal.

Considering the environmental toxicity and potential health concerns, advanced oxidation processes (AOP) of ultraviolet (UV) irradiation, ozonation, and hydrogen peroxide have been widely applied for ERY removal. Nakada et al. (2007) revealed that the combination of sand filtration/ozonation with activated sludge treatment gave an efficient removal (>90%) of ERY. Similarly, Kim et al. (2009) demonstrated that the combining UV with H_2O_2 removed more than 90% of ERY within the secondary effluent. Moreover, Fan and He (2011) found that the biodegradation of ERY improved significantly *via* carbon source (*e.g.*, glucose) and nutrients (N, P) addition. Despite AOP technologies showed promise for the removal of ERY, costs associated with nutrient augmentation and high-energy consumption severely limited its adoption.

In addition to AOP, the artificial recharge technique of soil aquifer treatment (SAT) was recently widely employed to treat those ERY polluted waterbodies for its relatively easy operation, low operational cost, and high efficiency (Pavelic et al., 2011). Several studies have reported the interactions between the removal trend of antibiotics/personal care products and the operational parameters of SAT systems. Arye et al. (2011) revealed that the average removal rate of carbamazepine was 30.56%–79.20% in 1.2 m depth SAT. Hua et al. (2003) found that greater than 90% removal of ibuprofen, iopromide, iohexol, and naproxen was achieved in biologically active sand columns. These studies principally focused on removal characteristics and transformation of typical antibiotics, while few studies focused on the aerobic or anaerobic degradation contribution of ERY following SAT treatment.

The objectives of this study were to (1) investigate the removal efficiency of ERY during SAT operation; (2) examine the contribution of aerobic biodegradation on bulk ERY reduction; and (3) identify putative degradation intermediates and the potential effects of dissolved organic matter (DOM) on ERY removal.

1. Materials and methods

1.1. Chemicals

ERY (CAS no. 114-07-8) used in this study was of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). High performance liquid chromatography (HPLC) grade methanol (99.9%; CAS no. 67-56-1), acetonitrile (99.9%; CAS no. 75-05-8) and triethylamine (CAS no. 121-44-8), analytical grade $NaHCO_3$ (CAS no. 144-55-8), $CaCl_2$ (CAS no. 10043-52-4), $MgSO_4{\cdot}7H_2O$ (CAS no. 10034-99-8), $CO(NH_2)_2$ (CAS no. 57-13-6), KH_2PO_4 (CAS no. 7778-77-0), K_2HPO_4 (99%; CAS no. 7758-11-4), et al. were purchased from Tianjin benchmark chemical reagent Co., LTD (Tianjin, China). Milli-Q water was used for all dilutions, samples and chemicals preparation, and final glassware cleaning. Stock solutions of 100 mg/L ERY were prepared in methanol and stored at 4°C (Gao et al., 2015).

1.2. Chemical characteristics of experimental soil sample and wastewater

Experimental soil samples were collected from the campus of Harbin Institute of Technology and were classified as sandy loam soil, its physicochemical characteristics are presented in Appendix A Table S1. The main compositions of the soil were found to be 56.83% SiO_2, 18.19% Al_2O_3, 8.92% CaO, 2.84% MgO, 7.73% Fe_2O_3, 0.89% TiO_2, 3.12% K_2O, and 0.86% Na_2O (W/W). The loss on ignition (LOI) was observed to be 5.4%. This soil samples had a pH of 8.1, organic carbon (OC) content of 3.63%, cation exchange capacity (CEC) of 16.3 cmol/kg, and Brunauer–Emmitt–Teller (BET) specific surface area of 45.0 m^2/g. The average pore size distributions of the soil were 49.4 Å.

During the acclimation period (3 months) and first-stage operation (4 months) of SAT, synthetic wastewater was chosen as influent, which was prepared according to OECD guidelines (1996) (in which, glucose 28.2 mg/L, amylum 28.2 mg/L, $NaHCO_3$ 13.5 mg/L, $CaCl_2$ 3.3 mg/L, KH_2PO_4 2.2 mg/L, NH_4Cl 30.6 mg/L, $MgSO_4{\cdot}7H_2O$ 10.2 mg/L, $CO(NH_2)_2$ 26.5 mg/L, and K_2HPO_4 2.8 mg/L). To clarify the effect of DOM existence in WWTP effluent on ERY removal, secondary effluent obtained from Taiping WWTP of Harbin was recharged simultaneously for a comparison; the recharged secondary effluent had a pH of 7.6 ± 0.3, chemical oxygen demand (COD) of 46.7 ± 4.8 mg/L, total organic carbon (TOC) of 14.7 ± 2.7 mg/L, dissolved organic carbon (DOC) of 12.5 ± 1.8 mg/L, UV-254 of 16.3 ± 1.7/m, nitrate of 5.4 ±2.7 mg N/L, ammonia of 9.3 ± 2.7 mg N/L, and total phosphorus of 2.3 ± 0.8 mg/L.

1.3. Operation of the lab-scale SAT columns

The SAT reactor consisted of two acrylic soil columns (50 cm in length, 10 cm in diameter), with top and bottom caps sealed with rubber gaskets, those columns were operated in-series at a flow rate of 15 mL/hr under gravity flow conditions (16 hr wetting cycle and 8 hr drying cycle). Air-dried soil samples described above were sieved through a 2 mm mesh screen and packed into the SAT column, then compacted to field density (with a density of 1.45 g/cm^3). The columns were operated in down flow mode and were therefore assumed to be predominantly unsaturated. Each SAT column was fitted with a water sampling port at the middle and bottom of the column. The average porewater velocities of the operated SAT columns were ranged from 2.31 to 2.44 cm/hr, demonstrating a residence time of about 10.2–10.8, 20.4–21.6, 30.6–32.4 and 40.8–43.2 hr for 25, 50, 75 and 100 cm depth sampling port, respectively. To prevent the growth of algae within the SAT column, all columns were wrapped with aluminum foil. Soil pore water extraction from different SAT soil layers was performed by inserting the porous plastic tubing of the Rhizon (capped with 0.45 μm nylon membrane) into the sampling ports firstly. At the other end of Rhizon soil moisture sampler,

a female Luer-Lock connector was attached to a disposable needle which was subsequently inserted into a vacuum tube to provide the required suction to extract soil solution (Vulkan et al., 2000). Specifically, all the samplers were washed by forcing 60 mL of 5% HNO_3 (CAS no. 7697-37-2) through the probe, followed by 60 mL of deionized water and then dried at 30°C before use. The samplers were placed in each water sampling port while the soils were being packed.

The above-mentioned lab-scale columns were firstly operated for approximately 3 months to acclimate *in situ* microorganisms under room temperature 25.0 ± 0.5°C, using synthetic wastewater as influent. Following steady-state operation (4 months), 10 μg/L ERY was spiked into the influent synthetic wastewater, and the ERY concentration at each depth (25, 50, 75, 100 cm) was detected every 5 days using HPLC. To investigate the contribution of aerobic biodegradation *versus* soil adsorption on the removal of ERY, the synthetic wastewater was also spiked with 2.0 mmol/L sodium azide (NaN_3; CAS no. 26628-22-8) to inhibit aerobic biodegradation (Xue et al., 2009), and then introduced into the SAT system. Before NaN_3 additive, above-mentioned SAT system was acclimated for another 4 months for the restarting/recovery, using synthetic wastewater as influent.

To evaluate the removal efficiency of ERY during WWTP secondary effluent recharging, ERY was separately added into the secondary effluent and DOM fractions as influent, and the performance of those SAT systems was evaluated. The operation of the SAT systems using secondary effluent as influent was similar to that of synthetic wastewater recharged columns.

1.4. Anaerobic/aerobic biodegradation test of ERY

To investigate the fate and behavior of ERY present in polluted aquifers, a sequential soil column system (SSCS) was constructed to simulate redox conditions from methanogenic, sulfate-reducing, denitrifying, to aerobic conditions according to Nay et al. (1999). Lovley (1997) stated that almost all chemicals could be efficiently biodegraded under one or more of the tested conditions. The SSCS system consisted of four glass tubes (Ø 2.5 cm, length 15 cm; Appendix A Fig. S1), which were wet-packed with washed, inoculated quartz sand (grain size 0.25 to 1.0 mm) and capped with Viton stoppers. Detailed information of the operational temperature, medium compositions, electron acceptors and carbon sources of the SSCS system is shown in the Supplementary materials.

1.5. Concentration analyzing of ERY

According to Xu et al. (2007), ERY within the SAT effluents and SSCS effluents was concentrated by solid phase extraction (SPE) using an Oasis HLB cartridge (500 mg, 6 mL, Waters Corporation, Milford, Massachusetts, USA). SPE cartridge was preconditioned with 2 mL methanol, 2 mL Milli-Q water and 2 mL Na_2EDTA (10 mmol/L, pH = 3; CAS no. 6381-92-6) in triplicate. After the conditioning step, 0.5 L filtered SAT effluent (pH adjusted to 3.0 with H_2SO_4, 3 mol/L) was loaded into the cartridge, using 0.11 mmol/L Na_2EDTA (CAS no. 6381-92-6) as chelating agent. Water samples were pumped into SPE Cartridges at a flow rate of 10 mL/min and then, rinsed with 5 mL of Milli-Q water prior the elution. After that, the ERY retained on the cartridges was eluted with 2 mL × 3 of methanol. The obtained ERY extracts were evaporated by a gentle nitrogen stream to a volume of approximately 20 μL, then re-dissolved in 1 mL acetonitrile for HPLC detection.

Concentration of ERY in the extracted samples was analyzed using HPLC with a fluorescence detector and detailed description could be found in the Supplementary materials. Because ERY could be easily transformed to ERY-H_2O during the SPE at acidic conditions, thus, ERY was measured as the sum of ERY-H_2O and ERY for the SAT effluent.

1.6. Liquid chromatography–Mass spectrometry (LC/MS analysis)

An Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA) equipped with an ABSciex API 5500 electrospray triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) was used for identification/quantification of the intermediates of ERY present in SAT effluent. Chromatographic separation was performed on a Hypersil-ODS C18 column (2.1 × 150 mm, internal diameter 5 μm). The mobile phase comprised 100% methanol (mobile phase A) and 0.06% formic acid (CAS no. 64-18-6) in Milli-Q water (mobile phase B), at a gradient flow of 0.3 mL/min. Mobile phase A was maintained at 30% for the first 1 min, then the percentage of phase B was linearly increased to 5% during the next 6 min, then to 70% in the following 11 min. Mass spectrometry detection conditions included a spray voltage of 4500 V; capillary temperature of 300°C; capillary voltage of 1500 V; sheath gas pressure of 337.8 kp and auxiliary gas pressure of 68.95 kp (nitrogen as sheath and auxiliary gases). MS data were recorded in the full scan mode (*m/z* 100–800).

Liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI–MS) source analysis was used for the identification of the potential intermediates of ERY, corresponding peaks to the chemical markers were identified in Liquid chromatography–quadrupole time of flight-mass spectrometry (LC–QTOF/MS) fingerprinting chromatograms. The working solutions of ERY in methanol solution were tested from 100 to 10,000 μg/L for calibration curves preparation and linearity determination, and the relative standard deviation and relative errors are calculated.

1.7. DOM fractionation and effect of their characteristics on ERY removal

DOM in the secondary effluent was fractionated using Amberlite XAD-8/XAD-4 resins into five fractions: hydrophobic acid (HPO-A), transphilic acid (TPI-A), hydrophobic neutral (HPO-N), transphilic neutral (TPI-N) and hydrophilic (HPI) fractions, as described in Wei et al. (2009). All fractionated organic samples were diluted to a same DOC level (12.5 mg DOC/L) and then adjusted to pH 7.0 ± 0.1, and 10 μg/L ERY was separately added into the HPO-A, TPI-A, HPO-N, TPI-N and HPI fractions. Subsequently, the ERY additive secondary effluent and fractionated organic solutions were separately pumped into another six SAT systems, and the removal efficiency of ERY and DOC was measured.

1.8. Chemical analyses

Electrical conductivity (EC) and CEC of the soil samples were determined according to EPA methods 9050 and 9081, respectively. Major cations, anions, and DOC were measured on inductively coupled plasma atomic emission spectroscopy (ICP-AES), ion chromatograph (Metrohm) and TOC-analyzer (Shimadzu V500). UV absorbance was measured with a Shimadzu UV-2550 ultraviolet–visible spectrophotometer (Shimadzu, Japan). All parameters were analyzed in triplicate, average values and standard deviations were calculated.

2. Results and discussion

2.1. Removal of ERY during the SAT system operation

Following an acclimation period of 3 months, actively growing microbial biomass on soil particles (Appendix A Fig. S2) revealed that the SAT systems were successfully started up. As shown in Fig. 1, the averaged removal rate of ERY decreased in the trend of 97.6% (day 5) > 95.7% (day10) > 90.1% (day 20) during the first 20 days operation, this was mainly ascribed to high adsorption capacity of ERY by the packed soil (Pan and Chu, 2016). ERY concentration of the SAT effluent kept relatively constant and exhibited a slight increase during the subsequent 25–120 days operation, with an average removal efficiency of 93.5%. Overall, bulk removal rate of ERY was much higher than that of carbamazepine (Arye et al., 2011) during steady-state operation of SAT, related to the lower inhibition rate of ERY on soil related gram-negative bacteria (Mao and Putterman, 1968; Pechere, 2001).

To distinguish the aerobic metabolic pathway of ERY from that of abiotic and anaerobic biodegradation, performance of the uninhibited and azide-inhibited SAT systems was compared. As shown in Fig. 2, ERY removal rate of the azide-inhibited SAT column decreased from 94.9% to 42.5% after the initial 30 days' operation, and subsequently kept constant. This could be explained by the fact that soil adsorption played a major role in ERY removal at the start-up period of SAT, especially under azide-inhibited condition. Additionally, soil pore water dilution in SAT column also contributed to ERY reduction (Essandoh et al., 2013). Effluent ERY concentration of the azide-inhibited SAT column was 6.29 μg/L under steady-state operation (35–120 days), resulting in an average removal rate of 37.1%. Xue et al. (2009) and Rattier et al. (2014) reported that sodium azide inhibition would eliminate aerobic microbial activity, thus the ERY reduction in azide-inhibited columns was ascribed to the combination of anaerobic biodegradation and soil sorption. Based on these analyses, we can conclude that aerobic biodegradation likely contributed as much as 60% to the bulk removal of ERY, suggesting that it was the dominant mechanism for ERY degradation.

2.2. Biodegradability of ERY under redox conditions in SSCS system

To investigate the behavior of ERY under aerobic/anaerobic circumstances in SAT system, redox conditions from methanogenic, sulfate-reducing, denitrifying, to aerobic conditions were constructed and the corresponding biodegradation process was analyzed. ERY biodegradation started under methanogenic condition, with about 5.9% of the added ERY (50 μg/L) was removed following an acclimation of 2 months, and it increased to 9.6% and 18.9% when the sulfate-reducing and denitrifying processes progressed (Fig. 3). Aerobically, ERY concentration continuously declined and was about 63.2% of its initial value. For comparison, ERY eliminated significantly in the denitrifying column and aerobic column after 4 months inoculation, and the corresponding removal rate of ERY was 6.7%, 12.6%, 26.2% and 67.9% when methanogenic, sulfate-reducing, denitrifying and aerobic processes progressed, respectively. Additionally, formation of a gray material with in the sulfate-reducing and denitrifying tubings indicated the formation of biofilm. When the inoculation period prolonged to 6 months, a residual 30.6% concentration of the ERY fed was released after the aerobic operation (69.4% ERY were transformed/biodegraded), and the removal distribution trends were similar to that observed from 4 months cultivation (Fig. 3). Our results further demonstrated that aerobic biodegradation was the key role for ERY removal during SAT operation, consistent with the findings of Grüenheid et al. (2005) and Abel et al. (2012), who observed a slower degradation of DOC within anoxic zone in soil passage/column as compared to aerobic zone.

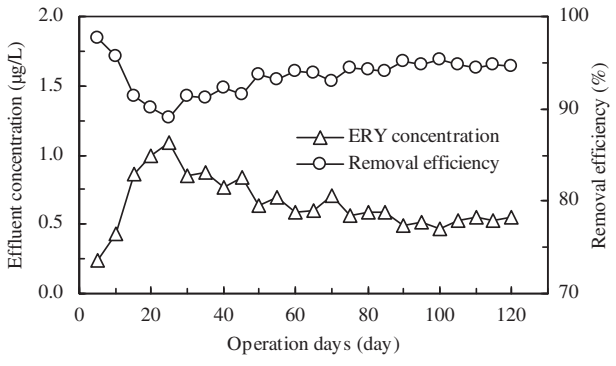

Fig. 1 – Effluent concentration and corresponding removal rate of ERY during the initial 120 days operation of soil aquifer treatment (SAT) system. ERY: erythromycin.

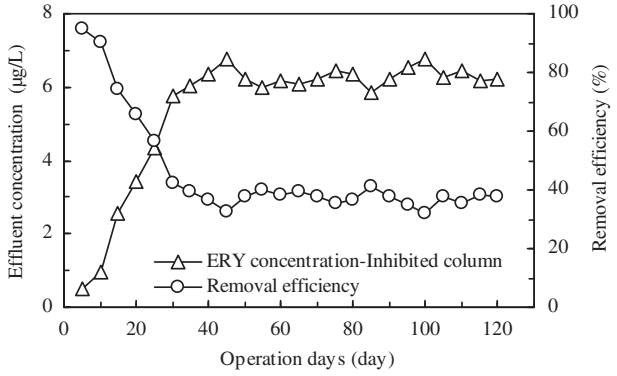

Fig. 2 – Effluent concentration of ERY and the corresponding removal rate of ERY during the initial 120 days operation of NaN_3 inhibited SAT column.

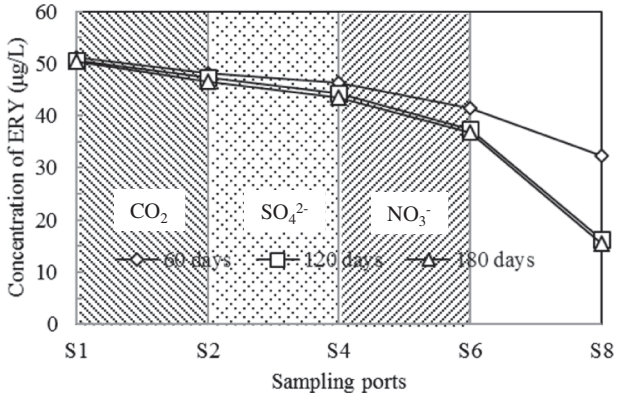

Fig. 3 – Biodegradation of ERY in sequential soil column system after 60, 120 and 180 days operation.

2.3. Depth distribution of ERY removal along the SAT column

Changes in column depth significantly affected the reduction of ERY during SAT operation. As shown in Fig. 4, about 45.9% of the bulk ERY was efficiently removed within the top 25 cm SAT column, and further increased to 65.4%, 83.3% and 92.9% when the soil layer depth increased to 50, 75 and 100 cm, respectively. In terms of the percentage contribution of the bulk removed ERY, the 0–25, 25–50, 50–75 and 75–100 cm soil layer contributed 49.4%, 21.0%, 19.3% and 10.3%, respectively. Undoubtedly, the top 25 cm soil layer played a key role in ERY reduction, above observations were similar to the DOM removal during lab-scale SAT operations (Xue et al., 2009; Wei et al., 2015). In comparison with the uninhibited columns, azide-inhibited SAT column exhibited a much higher effluent concentration of ERY, especially for the top soil layer. Specifically, the bulk ERY removal efficiency of the top 0–25 cm soil layer was 13.2% for the NaN_3 inhibited column, and increased to 22.0%, 30.9% and 37.4% when the depth reached 50, 75 and 100 cm, respectively. As a result, aerobic biodegradation contributed 71.2% of the bulk removal of ERY for the top 25 cm SAT layer, which gradually decreased to 54.8%, 50.9% and 31.5% for the 25–50, 50–75 and 75–100 cm soil layers. Obviously, increasing of the depth of soil column led to a simultaneous decrease of aerobic biodegradation, and negatively affected the bulk removal of ERY. These results were quite different from those reported by Maeng et al. (2011), who observed that anoxic conditions played a key role in the biodegradation of sulfamethoxazole.

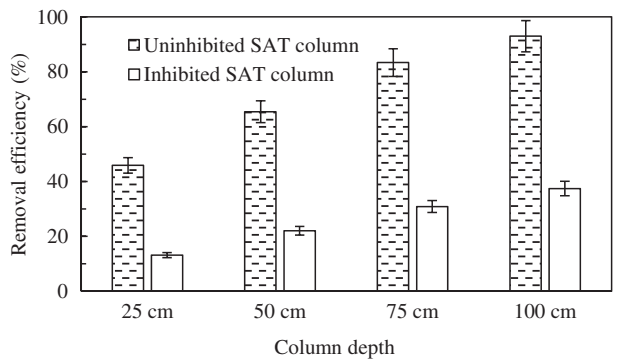

Fig. 4 – Removal rate distribution of ERY for different depth soil layers of the uninhibited and NaN_3 inhibited SAT column under steady-state operation.

2.4. Effect of DOM existence in secondary effluent on ERY removal during SAT operation

Numerous studies have demonstrated that the existence of DOM in secondary effluent can compete binding sites of soil in SAT system (Huang et al., 2014; Zhang et al., 2014), and negatively affected the bulk adsorption/degradation removal of antibiotics. As expected, removal rate of ERY declined significantly during the WWTP secondary effluent recharging (contained 10 μg/L ERY), revealed by a quite low ERY removal efficiency of 64.7% after 60 days operation (Fig. 5). Despite the degradation of ERY was typically relied on content of C, N, and P in secondary effluent (Fan and He, 2011), a more recent study by Gao et al. (2015) also pointed out a relatively high dosage of biodegradable carbon source (glucose, beef extract, and yeast) benefited the ERY degradation. Thus, a lower ERY removal rate observed from secondary effluent recharging might be ascribed to the relatively lower biodegradation rate of DOM as compared to that of glucose/amylum within synthetic wastewater.

The DOM in secondary effluent is a heterogeneous mixture of complex organic materials including hydrophilic materials, hydrophobic organics, carboxylic acids, and hydrocarbons. In general, the hydrophobic fraction of DOM has low polarity and high molecular weight, whereas hydrophilic organics exhibited high polarity and low molecular characteristics (Wei et al., 2009). DOM was the main carbon source available for ERY biodegradation during secondary effluent recharging; therefore their chemical characteristics likely influenced the removal efficiency of ERY. As shown in Fig. 6, recharging HPI as carbon source led to the highest ERY removal rate compared to the other four fractions, with 78.5% ERY reduction after 60 days operation (10 μg/L initial ERY concentration). Correspondingly, removal rate of ERY during the recharging with other four fractions decreased in the trend of TPI-N (75.5%) > HPO-N (69.6%) > HPO-A (65.5%) > TPI-A (63.2%). The highest removal rate of ERY during recharging with HPI was undoubtedly ascribed to its biodegradable characteristics (Xue et al., 2009). The recharging with HPO-A

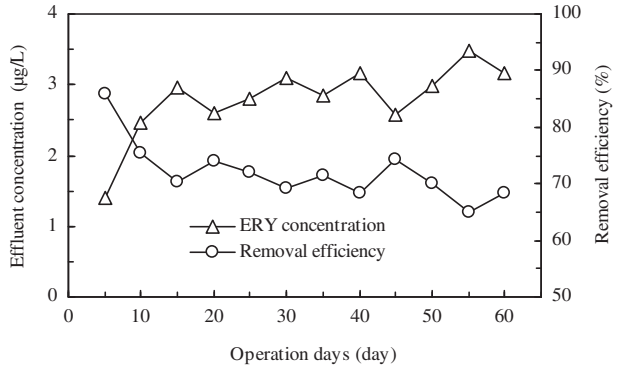

Fig. 5 – Effluent concentration and corresponding removal rate of ERY during the 60 days recharging with secondary effluent.

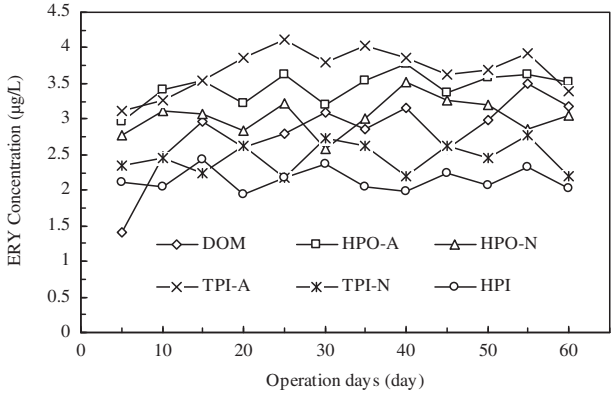

Fig. 6 – Removal efficiency of ERY during the 60 days recharging with dissolved organic matter fractions of HPO-A, HPO-N, TPI-A, TPI-N and HPI.

fraction (least biodegradable DOM fraction) exhibited a higher ERY removal efficiency in comparison with that of TPI-A, ascribing to the complexing interactions between ERY and the polymeric HPO-A under neutral pH condition. This result was quite similar to the observation that ERY exhibited a surfactant-like structure and could be easily removed *via* hydrophobic interactions (Kummerer, 2009; Le-Minh et al., 2010).

2.5. *Biodegradation and the intermediates of ERY during SAT column operation*

Identification of the main degradation intermediates of ERY generated after SAT operation was accomplished in order to propose a degradation pathway. ERY contains a 14-member macrocyclic lactone ring with 10 asymmetric centers and two sugars (L-cladinose and D-desosamine), making it a typical aromatic structure and refractory characteristic. Analysis by LC/MS allowed for the detection of six main intermediates of ERY (Table 1), and a possible degradable pathway is illustrated in Fig. 7.

Starting with ERY, there are three possible pathways for its further biodegradation during SAT operation. The first one is hydrolysis of ERY, yielding the intermediates with a *m/z* ratio of 752. The weak alkaline conditions of the experimental soil (pH 7.74) and abundance of pore water might be the main reasons for the formation of this new intermediate. Simultaneously, ERY could also lose a water molecule and produce the new intermediate of $C_{37}H_{65}NO_{12}$ (*m/z* ratio of 716). Previous studies have reported that ERY exhibited strong sensitivity to pH, and inclined to lose a water molecule once the pH was less than 7.0 (Yang and Carlson, 2004); thus the production of $C_{37}H_{65}NO_{12}$ may be associated with anaerobic reactions (resulting in acidic pH condition) within the interpores of packed soil (Ye et al., 2012). The secondary degradation pathway corresponds to the opening of the 14-member macrocyclic lactone ring and yields the stereoisomers of $C_{21}H_{40}O_9$ formula (*m/z* ratio of 436) *via* path 2 in Fig. 7. Our results demonstrated that L-cladinose and D-desosamine bound onto the ERY could be both degraded during the SAT operation. Finally, a third degradation route through the formation of $C_{29}H_{50}O_{10}$ (14-member macrocyclic lactone ring and L-cladinose) was proposed during the SAT operation, due to the loss of D-desosamine group. The presence of fragments at *m/z* 158 ($C_8H_{16}NO_2$), resulting from a decarboxylation reaction, confirmed this proposal. Overall, the D-desosamine group in ERY could be more easily biodegraded during the SAT operation in comparison to that of L-cladinose. A further decarboxylation reaction of D-desosamine ($C_8H_{16}NO_2$) yielded the formation of intermediates $C_6H_{14}NO$, with a *m/z* ratio of 116. Furthermore, the intermediates of $C_8H_{16}NO_2$ and $C_6H_{14}NO$ exhibited a simple chemical structure, which could be easily converted to CO_2 and H_2O during long-term operation of SAT systems. Since the side chains of macrolide antibiotics directly affect the interaction of those drugs with specific ribosomal ribonucleic acid (rRNA) residues, while the central macro-lactone ring has little influence (Dunkle et al., 2010; Kannan and Mankin, 2011), thus the conversion of ERY to intermediate of 14-member lactone ring, L-cladinose and D-desosamine undoubtedly lowered the toxicity of ERY to the biomass after SAT operation.

Table 1 – Structures of the identified intermediates in the degradation process of erythromycin (ERY).

Retention time (min)	4.90	7.34	11.69	15.90
Mass-to-charge ratio	116 158	436	558	716 752
Formula	$C_6H_{14}NO$ $C_8H_{16}NO_2$	$C_{21}H_{40}O_9$	$C_{29}H_{50}O_{10}$	$C_{37}H_{65}NO_{12}$ $C_{37}H_{69}NO_{14}$

3. Conclusions

The following conclusions can be drawn from the results of this study: (1) The average removal rate of ERY during steady-state of SAT operation was 92.9%, which was much higher than that of 37.1% in NaN_3 inhibited SAT column. Aerobic biodegradation contributed approximately 60% of the bulk ERY removal during 1.0 m depth SAT operation. SSCS exhibited a similar trend and was predominant by aerobic biodegradation. (2) Top 25 cm soil layer played a key role in ERY reduction in SAT system when recharged with synthetic influent, contributing about 49.4% of the bulk ERY removal. Aerobic biodegradation was responsible for 71.2% of the bulk ERY removal for the top 25 cm soil layer and declined to 54.8%, 50.9% and 31.5% for the 25–50, 50–75 and 75–100 cm soil layers. (3) DOM existence in recharged influent competed for the binding sites in soil and negatively deteriorated SAT performance, revealed by a low ERY removal rate of 64.7% under secondary effluent recharging. HPI could be preferentially utilized as carbon source and was beneficial for ERY removal. The removal rate of ERY (10 μg/L) during different DOM fractions recharging decreased in the trend of HPI (78.5%) > TPI-N (75.5%) > HPO-N (69.6%) > HPO-A (65.5%) > TPI-A (63.2%). (4) The opening of 14-member macrocyclic lactone ring and lose of L-cladinose and D-desosamine bound to ERY yielded six intermediates during ERY recharging. Both L-cladinose and D-desosamine bound to ERY were efficiently destructed during SAT operation, where D-desosamine group could be more easily degraded.

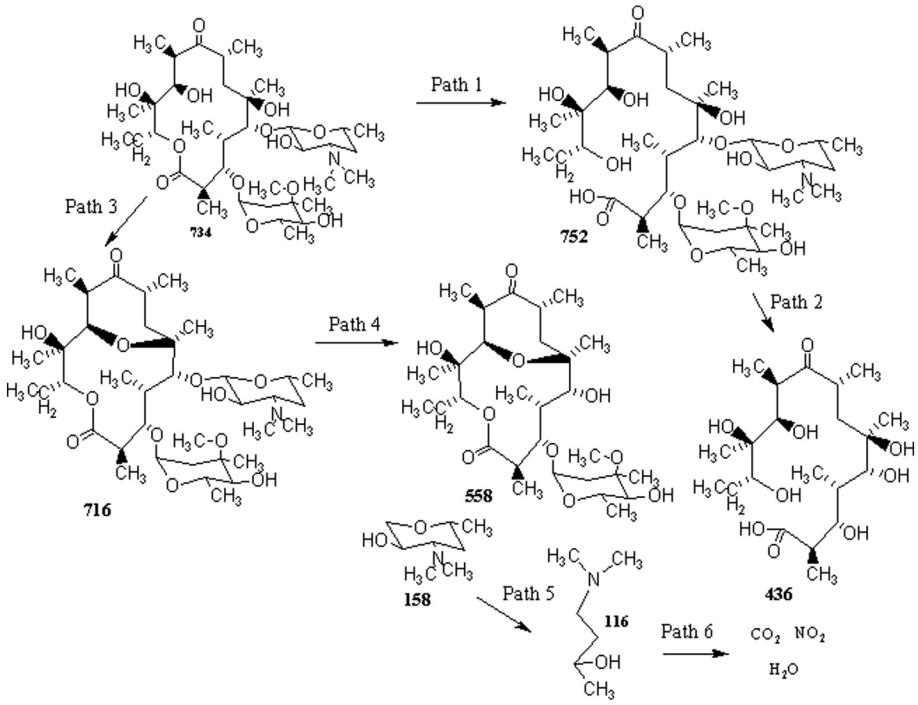

Fig. 7 – Proposed degradation pathway of ERY during SAT operation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.08.004.

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