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Struvite crystallization induced the discrepant transports of antibiotics and antibiotic resistance genes in phosphorus recovery from swine wastewater☆

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ABSTRACT

Struvite ($MgNH_4PO_3 \cdot 6H_2O$) crystallization is one of important methods of phosphorus recovery from wastewater. As to livestock wastewater, the high-strength occurrence of antibiotics and antibiotic resistance genes might induce struvite recovery to spread antibiotic resistance to the environment. However, limited information has been reported on the simultaneous transport of antibiotics and ARGs in struvite recovery. In the present study, tetracyclines (TCs) and tetracyclines antibiotic resistance genes (ARGs) were selected as the targeted pollutants, and their discrepant residues in struvite recovery from swine wastewater were investigated. TCs and ARGs were obviously detected, with their contents of 4.88−79.5 mg/kg and 6.99×10^7–2.14×10^{11} copies/g, notably higher than those of TCs 0.550−1.94 mg/kg and ARGs 3.98×10^4–5.66×10^7 copies/g obtained from synthetic wastewater. The correlational relationship revealed that predominant factors affecting TCs and ARGs transports were different. Results from network analyses indicated that among the total edges, the negative correlations between TCs and ARGs predominately occupied 18.0%. The redundancy analysis revealed that mineral components in the recovered products, including struvite, K-struvite and amorphous calcium phosphate, coupling with organic contents, displayed insignificant roles on TCs residues, where heavy metals exerted positive and remarkable functions to boost TCs migration. Unexpectedly, mineral components and heavy metals did not displayed significant promotion on ARGs transport as a whole.

1. Introduction

Due to rapid economic growth and urbanization in China over the past few decades, a large number of swine farms have emerged to supply pork to meet people's demand (Cheng et al., 2019). According to the statistics, the annual production of pigs in China has reached 702 million (NBSPRC, 2018). Subsequently, numerous nitrogen and phosphorus have been discharged from livestock wastewater and intensify the environmental pollution, which has triggered extensive concern in public (Lin et al., 2014). From another aspect, such large amount of nitrogen and phosphorus existing in livestock wastewater has great potential for nutrient recovery, such as struvite ($MgNH_4PO_4 \cdot 6H_2O$) crystallization, which has been considered as an important means of retarding the scarcity of phosphorus rock worldwide (Agrawal et al., 2018; Yan et al., 2018). Struvite is a slow release fertilizer with high contents of phosphate and ammonium, which can be used directly in agriculture.

Antibiotics are widely used in animal feeding to prevent diseases and promote animal growth. However, it has been reported that about 30−90% of antibiotics are discharged in the form of parent compounds or metabolites into feces and urine, since antibiotics are hard to be assimilated in the animal guts (Ma et al., 2018). For instance, the highest total tetracyclines (TCs) and sulfonamides (SAs) concentrations detected in swine wastewater were 317 μg/L and 686 μg/L, respectively (Cheng et al., 2018). Besides, antibiotics could only be partially removed in wastewater treatment systems (Liu et al., 2014; Yao et al., 2017). After the anaerobic

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digestion treatment, the removal rate for antibiotics in swine wastewater could range from 11% to 86%, indicating that high concentrations of antibiotics still remained in the treated wastewater (Gros et al., 2019).

Antibiotic resistance genes (ARGs) have been defined as the emerging environmental pollutants worldwide. The proliferation and pervasion of ARGs have been regarded as a big concern for public health as reported by World Health Organization (Pruden et al., 2006). ARGs could be transferred to recipient bacteria through conjugation (from a donor cell to a recipient cell through mobile genetic elements), transformation (uptake of extracellular ARGs) and transduction (bacteriophage as the carrier) (Wang et al., 2018b). Such transfer behaviors can result in spreading of antibiotic resistance and the failure of medical treatment to human (Lan et al., 2019). It has been reported that approximate 700,000 people die of antibiotic resistance every year worldwide, and this amount might be up to 10 million by 2050 if effective controlling methods of antibiotic resistance cannot be proposed (Willyard, 2017). With regard to pig farming, literature revealed that various kinds of ARGs exist extensively in swine wastewater, including tetracyclines resistance genes, sulfonamide resistance genes and quinolone resistance genes, and their concentrations varied from 2.20×10^4 copies/L to 1.17×10^9 copies/L (Wang et al., 2016; Yuan et al., 2018).

The frequent exposure of antibiotics can influence microorganisms in the environment, thus resulting in changes of microbial activities and community compositions (Szymańska et al., 2019). On the other hand, long-term exposure to antibiotics will promote the pervasion of antibiotics resistance among bacterial including pathogenic bacteria and spread ARGs to the environment, which thus pose risks to the health of human and animals (Magwira et al., 2019). It has been reported that the transport of antibiotic and ARGs in the environment were significantly affected by the environmental factors, such as temperature and pH (Ye et al., 2017; Zhang et al., 2019) and the properties of wastewater, including the metals, ions, organic matters etc (Ji et al., 2012; Wang et al., 2019a; Ye et al., 2020). With regards to struvite recovery from wastewater, relevant information on antibiotics and ARGs was limited. In struvite crystallization, the wastewater would undergo drastic reduction of ion strength under alkaline conditions and the subsequent formation of crystallite struvite particles. Previous researches revealed that TCs and ARGs could be respectively detected in the struvite recovery products (Ye et al., 2020; Wang et al., 2018a; Zhai et al., 2019), which suggested that the transport of antibiotics and ARGs in struvite recovery from wastewater might simultaneously occur. However, little literature has reported on this issue. In this study, tetracyclines (TCs) and tetracyclines antibiotic resistance genes, the most broad-spectrum antibiotics (Cheng et al., 2019; Ma et al., 2018) and frequently detected ARGs (including *tet*A、*tet*M、*tet*G、*tet*Q、*tet*X and *tet*32) in swine wastewater (Wang et al., 2016; Yuan et al., 2018), were selected as the typical target pollutants, and struvite recovery from wastewater was performed. The simultaneous residues of TCs and ARGs in the recovered product were examined. Furthermore, the statistical methods, including **Pearson correlation analysis,** redundancy analysis and network analysis, were applied to screen out discrepant factors which significantly contributed to TCs and ARGs migration.

2. Materials and methods

2.1. Chemicals and standards

Tetracyclines (TCs) including tetracycline (TC), oxytetracycline (OTC),chlortetracycline (CTC) and doxycycline (DXC) were purchased from the Alladin Inc. (USA). Tetracycline-D6 (TC-D6), as an internal standard for analysis, was purchased from the Tononto Research Chemicals Inc., Canada. Oasis HLB cartridges (size of 200 mg, 6 mL), which were used in the process of solid phrase extraction for antibiotic analyses, were brought from the Waters (Milford, USA). Swine wastewater was obtained from the anaerobically digested effluent in seven intensive swine farms, which were located in Longyan City, Fujian Province, China, and the characteristics of seven swine farms were presented in Table S1. The parameters of swine wastewater in seven swine farms were shown in Table S2. The initial concentrations of TCs and ARGs were $12.0–4.23 \times 10^3$ μg/L and $2.14 \times 10^8–1.32 \times 10^{13}$ copies/L, respectively, which were exhibited in Fig. S1 and Fig. S2.

Other chemical agents used for struvite precipitation, including $MgCl_2$ and NaOH were chemically pure and provided by the Xi Long Co. (China). Ultrapure water was obtained from a Milli-Q water purification system (18.2 ΩM cm) from Millipore (Boston, USA). The plasmids containing specific genes (i.e. *tet*A, *tet*M, *tet*Q, *tet*G, *tet*X, *tet*32) for absolute quantification were produced by Shanghai Meiji Biotechnology Company (Shanghai, China).

2.2. Experimental design and setup

As described above, wastewater samples obtained from seven swine farms were directly subjected to struvite precipitation. It should be pointed out since phosphate in some wastewater samples was not sufficient for fully achieving struvite precipitation, a certain amount of phosphate stock solution was added into wastewater to control initial PO_4^{3-}-P at approximate 100 mg/L. The concentrations of PO_4^{3-}-P and total phosphorus (TP) in the wastewater after adding phosphate stock solution was showed in Table S2. Desired volume of Mg^{2+} solution was dosed to maintain the molar ratio of Mg:P at 1.2:1, and pH value was set to 9.5 by dosing 10 mol/L NaOH to achieve struvite precipitation. During struvite formation, the liquor was agitated with the speed of 200 rpm for 45 min. After that the precipitates were settled for 60 min, and the mixture was centrifuged for solid-liquid separation. The recovered solids were further subjected to freeze-drying, and stored at −18 °C before analyses. The recovered solid samples from swine wastewater of seven swine farms through struvite crystallization were defined as S1-7, respectively.

In order to screen out the effects of the property of real wastewater on TCs and ARGs migration in struvite recovery, two blank experiments were conducted to achieve struvite precipitation by using synthetic wastewater. Stock solutions containing NH_4^+, PO_4^{3-} were respectively prepared by dissolving NH_4Cl, $MgCl\cdot6H_2O$, and $NaHPO_4$ into the sterilized waters. The concentrations of TCs and the abundances of ARGs stock solution were 300 mg/L and 10^{16} copies/L, respectively. The quantities of NH_4^+, PO_4^{3-} and Mg^{2+} for struvite precipitation were adjusted as the same levels as real wastewater. Desired amount of TCs and ARGs were respectively dosed into beakers to keep initial TCs 50 μg/L and ARGs 10^{10} copies/L. Struvite precipitation was performed by controlling the same operational conditions to those in real wastewater. The recovered struvite from two blank experiments was named as Pure struvite 1–2, respectively.

2.3. Analytical methods

2.3.1. Common methods

Ammonia nitrogen, phosphate, nitrate, total nitrogen and total phosphorus were measured by the standard methods (APHA, 1998). Metal were determined by inductively coupled plasma atomic emission spectrometer (Optima 7000DV, PerkinElmer, USA). Ion chromatography (Aquion ICS, Thermo Fisher, USA) was used for the determination of sulfate and chloride. TOC analyzer (TOC-Veph, Shimadzu, Japan) was employed to determine organic carbons. The solids produced in struvite precipitation were grinded and then were subjected to X-ray diffractometer (X'Pert PROMPD, PANalytical Ltd., Holland) for crystallite analyses.

2.3.2. Antibiotics assay

Before antibiotic assay, solid-phase extraction (SPE) method (Xu et al., 2013) was employed as the pretreatment method, in which the Oasis HLB cartridge (200 mg, 6 mL, waters, Milford, USA) was used to extract antibiotics. After that, the liquid chromatography/tandem mass spectrometry (LC-MS/MS) (ABI3200 QTRAP, USA) in combination with a Phenomenex Kinetex Symmetry C18 column (4.6 mm × 100 mm) was adopted to determine individual TCs concentrations. The mass spectrometry system fitted with electrospray ionization (ESI) source was run in the positive mode with desolvation temperature at 300 °C and capillary voltage 5.5 kV.

The detection of antibiotics with LC-MS/MS were examined for recovery rate, detection limits and the quantification for the instrument by the use of calibration curves which has been described in the literature (Xia et al., 2016; Ye et al., 2017).

2.3.3. ARGs extraction and quantification

FastDNA Spin Kit (MP Biomedicals, USA) was used to extract DNA from sample (Chen et al., 2017; Zhang et al., 2017), with the procedure based on the manufacturer's instructions. The determinations of purity and concentration of extracted DNA were carried out by using spectrophotometry analysis (NanoDrop-1000, NanoDrop Technologies, Wilmington, DE).

According to previous studies, *tet* ARGs, represented as tetracyclines antibiotics resistance genes, were predominantly detected in the swine wastewater (Chen et al., 2017; Lan et al., 2019). In this study, typical *tet* ARGs, including *tet*A, *tet*M, *tet*G, *tet*Q, *tet*X and *tet*32, were selected as the targeted genes. In order to detect targeted genes, these genes required to be amplified by using Quantitative Real-time PCR, namely qPCR. Briefly, desired amount of LightCycler 480 SYBR Green I Master (Roche, Switzerland), forward/reverse premiers (Meiji Biotechnology Company, China) were added in the PCR process. The qPCR procedures were conducted as described by previous literature (Sui et al., 2016). The abundance of ARGs was determined using QuantStudio 6 Flex Real-Time fluorescence PCR System (Applied Biosystems, USA). Information about primers used in qPCR, including sequences and annealing temperature of primers and so on was listed in Table S3 (Aminov et al., 2001; Apley et al., 2012; Diehl and Lapara, 2010; Ng et al., 2001; Zhu et al., 2013). Standard curves containing seven points for qPCR were generated by dilluting the stock solution of plasmid carrying target genes, and R2 values were all higher than 0.99, as showed in Fig. S4. The melting curves of ARGs properties were given in Fig. S5.

2.4. Statistical analysis

Pearson correlation analysis combining with SPSS software (version 19) was implemented to evaluate the factors affecting TCs and ARGs residues in the recovered solids. The redundancy analysis of environmental variables and the abundance of ARGs were performed by using CANOCO 5.0 software (Microcomputer Power, USA). The network analysis was carried out by using RStudio with "picante" package and Cytoscape 3.6.1 software to estimate the correlations among TCs, ARGs, wastewater properties and struvite precipitation.

3. Results

3.1. TCs and ARGs residues in the recovered solids

TCs contents in the struvite products obtained from real wastewater were illustrated in Fig. 1, where TC was 6.51−53.4 mg/kg, OTC 6.42−79.5 mg/kg, CTC 4.88−31.1 mg/kg, DXC 6.17−30.6 mg/kg, respectively. Comparatively, the solids recovered from synthetic wastewater possessed lower TCs contents (Fig. 2), with TC at 1.94 mg/kg, OTC at 1.43 mg/kg, CTC at 1.43 mg/kg and DXC at 0.550 mg/kg, respectively. Hence, it could be indicated that other components consisted in the real wastewater, such as organics, metal compositions, might boost TCs migration from the wastewater to the recovered products.

Fig. 3 and Fig. 4 displayed ARGs abundances in the product solids recovered from real wastewater and synthetic wastewater, respectively. The targeted ARGs (*tet*A, *tet*M, *tet*G, *tet*Q, *tet*X, *tet*32) were detected in all solids. According to the gene abundance in axis scales, the tetracycline resistant genes in products recovered from real wastewater were distinctly higher than those obtained from synthetic wastewater. There existed ARGs in the pure recovered products suggested that struvite crystals could adsorb a certain amount of ARGs. Comparatively, the obviously higher ARGs in the solids obtained from real wastewater suggested that other components in the wastewater might augment ARGs migration from the wastewater to the recovered products.

3.2. Other components existed in the solids

In order to screen out the discrepant factors which might influence TCs and ARGs transport during struvite recovery from real wastewater, the compositions in the recovered products were also determined. The precipitated components were firstly detected (Table 1), which were based on the methods described in our previous researches (Shen et al., 2015; Ye et al., 2011). It could be seen that struvite accounted for large proportions in the attained solids, which was also verified by XRD spectra (Fig. S1). In addition to struvite, there were other minerals detected in the precipitates, including K-struvite ($MgKPO_4 \cdot 6H_2O$) and amorphous calcium phosphate (ACP, $Ca_3(PO_4)_2 \cdot xH_2O$). It should be noted that organic matters did obviously exist in the recovered solids, indicating that organic matters might play some functions of the transportation of TCs and ARGs from wastewater into recovered solids. Further experiments with strict design are needed to investigate the roles of organic matters.

It has been confirmed that heavy metals exert important functions on TCs and ARGs migrations in the environment (Hu et al., 2017; Pulicharla et al., 2017). In the present study, various heavy metals in recovered solids were also analyzed, as presented in Fig. 5. High contents of Zn with the range from 366 mg/kg to

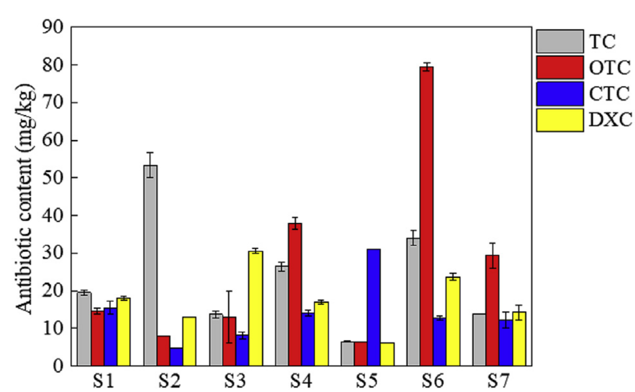

Fig. 1. Antibiotics contents in the struvite solids obtained from seven swine farms. S1, S2, S3, S4, S5, S5 and S7 represented the recovered solid samples from swine. wastewater of seven swine farms through struvite crystallization, respectively.($N = 3$, error bars indicated standard deviation).

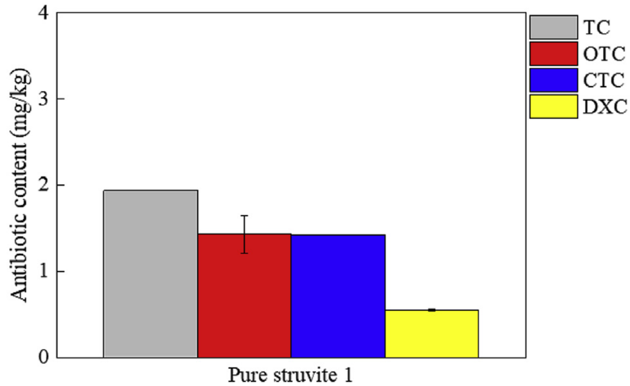

Fig. 2. Antibiotic contents in the solids recovered from synthetic wastewater. ($N = 3$, error bars indicated standard deviation).

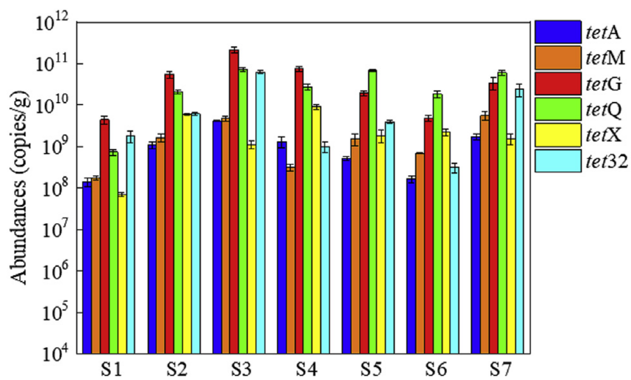

Fig. 3. Absolute abundance of ARGs in the solids recovered from real wastewater obtained from different swine farms. S1, S2, S3, S4, S5, S5 and S7 represented the recovered solid samples from swine wastewater of seven swine farms through struvite crystallization, respectively. ($N = 3$, error bars indicated standard deviation).

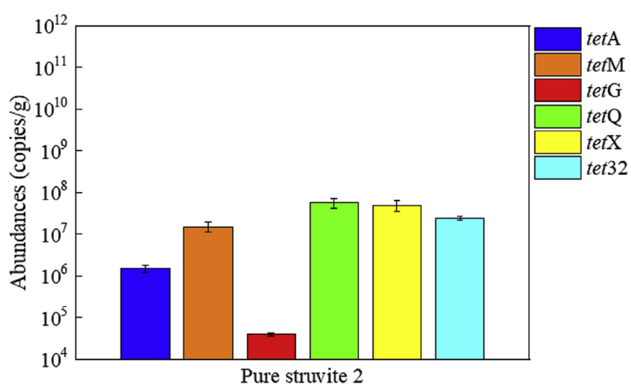

Fig. 4. Absolute abundance of ARGs in the solids recovered from synthetic wastewater. ($N = 3$, error bars indicated standard deviation).

Table 1
Compositions of the solids recovered from swine wastewater.

Sample	Components in the recovered solids (mg/g)			
	Struvite[a]	K-struvite[b]	ACP[c]	OC[d]
S1[e]	563 ± 35.8	299 ± 2.45	17.6 ± 0.430	86.2 ± 1.38
S2	547 ± 24.5	43.5 ± 0.780	201 ± 1.05	129 ± 0.580
S3	594 ± 6.47	38.1 ± 0.250	149 ± 0.510	141 ± 0.590
S4	665 ± 54.0	62.7 ± 1.91	171 ± 0.550	39.3 ± 0.670
S5	353 ± 10.4	123 ± 3.02	151 ± 0.780	286 ± 1.42
S6	487 ± 10.6	68.2 ± 1.39	299 ± 1.38	103 ± 1.12
S7	392 ± 64.7	91.4 ± 1.09	161 ± 1.77	190 ± 1.88

[a] Struvite, $MgNH_4PO_4 \cdot 6H_2O$.
[b] K-struvite, $MgKPO_4 \cdot 6H_2O$.
[c] ACP, amorphous calcium phosphate, $Ca_3(PO_4)_2 \cdot xH_2O$.
[d] OC, organic carbon.
[e] S1, S2, S3, S4, S5, S5 and S7 represented the recovered solid samples from swine wastewater of seven swine farms through struvite crystallization, respectively.

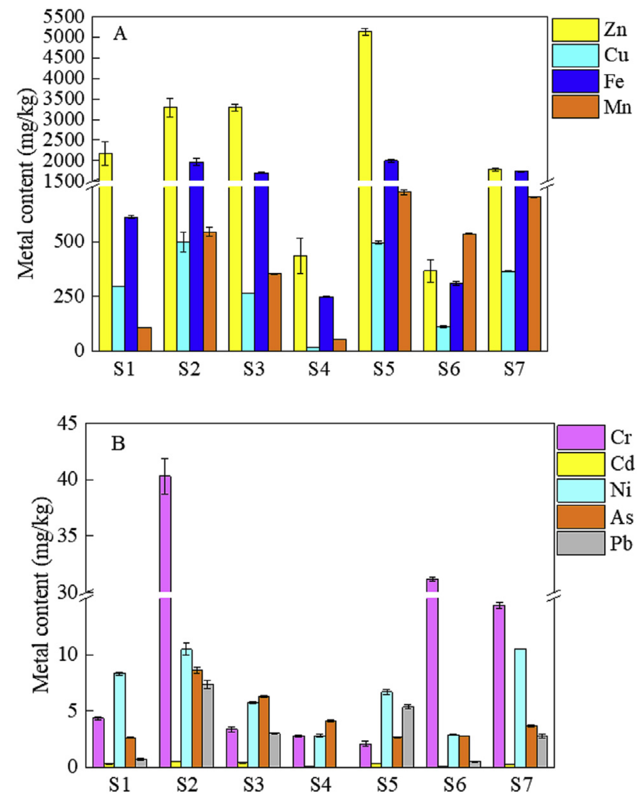

Fig. 5. Heavy metal contents in the recovered solids generated from real wastewater. A for the metals of Zn, Cu, Fe and Mn; B for the heavy metals of Cr, Cd, Ni, As and Pb. S1, S2, S3, S4, S5, S5 and S7 represented the recovered solid samples from swine. wastewater of seven swine farms through struvite crystallization, respectively. ($N = 3$, error bars indicated standard deviation).

3.29×10^3 mg/kg were detected. As to Cu、Fe and Mn, they were found obvious residues with the range at 16.5–498 mg/kg, 249–2.00×10^3 mg/kg and 53.4–727 mg/kg, respectively. Heavy metals can be regularly detected in swine wastewater discharged from the intensive pig farming, since their compounds (like ZnO and $CuSO_4$) are commonly used as growth promoters for animal feeding (Shen et al., 2015). Their residues in phosphate recovery from wastewater have also been reported by previous studies, which could be ascribed to their co-precipitation with struvite precipitation (Shen et al., 2015; Tang et al., 2019; Yee et al., 2019).

4. Discussion

4.1. Correlation between TCs and ARGs

Struvite crystallization has been widely employed as an important means to recover phosphorus from wastewater (Agrawal et al., 2018; Yan et al., 2018). However, results from the present study revealed that the simultaneously high-strength residues of antibiotics and ARGs in the recovered products, which might pose directly pharmaceutical risks to the environment. As shown in Figs. 1 and 3, TCs and ARGs residues in the recovered products reached to 4.88–79.5 mg/kg and 6.99×10^7–2.14×10^{11} copies/g.

On the basis of previous researches, such high levels of pollutants in the recovered struvite may exert high selective-pressure on soil microorganisms and transfer and spread ARGs to the crops (Chen et al., 2017; Lou et al., 2018). It should be noted that TCs and ARGs residues in the solids obtained from synthetic wastewater kept low concentrations, with the respective ranges at 0.550–1.94 mg/kg and 3.98×10^4–5.66×10^7 copies/g, which hinted that the properties of wastewater and the operational conditions of struvite precipitation were the key factors which boosted TCs and ARGs migration. Further examination on the correlation between TCs and ARGs was conducted by using the Pearson Analysis method, as showed in Table 2. It was interesting that insignificant relationship between TCs and ARGs in the solids ($P > 0.05$) was observed. Such outcome suggested that predominant factors affecting TCs and ARGs transports from the wastewater to the precipitates were different.

4.2. TCs transport

Redundancy analysis (RDA) was applied to analyze the relationship among TCs contents and other compositions in the solids (Fig. 6), which could indirectly reflect the influences of wastewater property and struvite precipitation. The concentrations of Mg, K, Ca, NH_4–N, P and C could be further calculated as the contents of struvite (elements Mg, N and P), K-struvite (elements Mg, K and P), amorphous calcium phosphate (ACP) (element Ca and P) and organic matters (element C) in the recovered solids, with their values displayed at Table 1. Results of Decision Curve Analysis with the gradient length of axis1 was 1.5 < 3.0, revealing that the application of RDA was valid. According to Fig. 6, the axes could explain most of the total variance of TCs contents in the recovered products. As illustrated in Table S4, the metal of Mg, Ca and K occupied 14.7% of the total variation, which hinted that mineral components in the solids, including struvite, K-struvite and ACP, did not play significant roles on TCs residues in the precipitates. Such outcomes were also verified by the results of synthetic wastewater, where TCs contents in the final solid phase were 0.550–1.94 mg/kg, extremely lower than those obtained from real wastewater (4.88–79.5 mg/kg). With regard to C, it was a bit surprise that its contribution was just 0.1% of the total variables. As reported by previous researches, organic matters could easily complex with TCs and boost TCs migration (Ye et al., 2018; Lou et al., 2015). Considering that all solid samples contained high organic contents (TOC 39.3–286 mg/g) as presented in Table 1, the distinction of organics effects on TCs residues in all samples could not easily emerge. pH was employed in RDA procedure to describe struvite precipitation conducted under alkaline conditions. As shown in Table S4, pH exerted the positive effects and owned 29.2% of the total variation of TCs, which from other aspect confirmed struvite crystallization was the key factor to boost antibiotic migration in the nutrient recovery process. The combination of various heavy metals, including Zn, Cu, Fe, Mn, Cr, Cd, As, Pb, Ni, explained 54.1% of the total TCs variation (Fig. 6). As reported, TCs were characterized by three kinds of dissociated functional groups, including tricarbonyl group, dimethylamine group and phenolic diketone moiety (Pulicharla et al., 2017). They could easily complex with different heavy metals or organics to form ternary chelates through different mechanisms, such as electrostatic interaction, hydrogen boding electron donor-acceptor, π-π dispersion (Pulicharla et al., 2017; Wang et al., 2019b). Therefore, the effects of heavy metals on TCs transport in struvite recovery from wastewater should not be ignored.

Table 2
Correlation analysis on the ARGs abundance and TCs residues concentrations in the solids recovered from real wastewater.

Item	*tet*A	*tet*M	*tet*Q	*tet*G	*tet*X	*tet*32
TC[a]	−0.241[b]	−0.371	−0.626	−0.140	0.542	−0.357
OTC	−0.342	−0.257	−0.348	−0.342	0.117	−0.332
CTC	−0.411	−0.244	0.317	−0.408	−0.204	−0.352
DXC	0.579	0.221	−0.020	0.637	−0.195	0.622
TTC	−0.352	−0.390	−0.502	−0.288	0.255	−0.374

[a] TC, OTC, CTC, DXC and TTC represent tetracycline, oxytetracycline, chlortetracycline, doxycycline and total tetracyclines, respectively.
[b] Value of Pearson correlation coefficient (r) was showed in each cell. All values suggested statistical insignificance ($P > 0.05$).

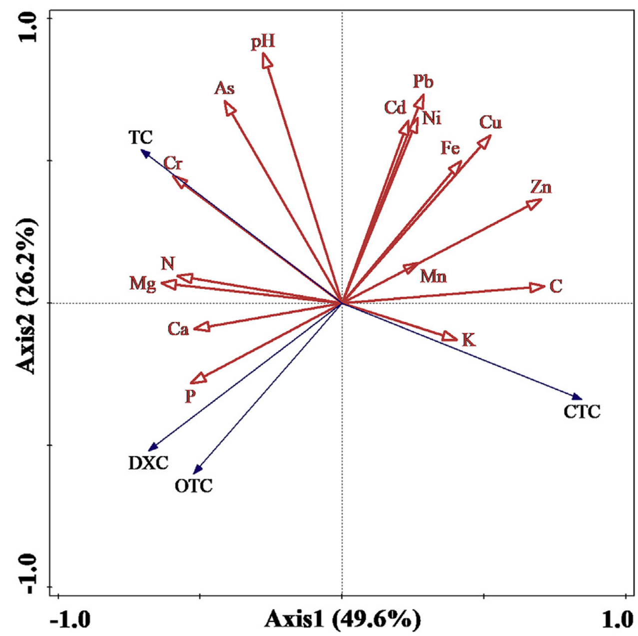

Fig. 6. Redundancy analysis of the correlation among four kinds of tetracyclines and other components in the solids recovered from real wastewater. TCs are expressed by the blue solid arrows, and other factors are expressed by red hollow arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.3. ARGs transport

It has been reported that antibiotics and heavy metals could exert selective pressure on the proliferation and pervasion of ARGs through several mechanisms, including co-resistance, cross-resistance and co-regulation (Wang et al., 2019a; Zhao et al., 2019), which hinted that the behavior of antibiotic and ARGs might not have good consistence (Mckinney et al., 2010), and the methods of correlation analysis were inappropriate to evaluate the relationships among ARGs, antibiotics and heavy metals. In this study, the network analysis was constructed to explore the significant relationship among ARGs, TCs, solid components, metals and reaction pH. As shown in Fig. 7, the co-occurrence network consisted of 26 nodes (6 ARGs, 4 TCs, 6 components, 9 heavy metals, and reaction pH) and 126 links. Unexpectedly, negative correlations were observed predominately between ARGs and TCs. As illustrated in Fig. 7, the links connecting TCs and ARGs accounted for 22.5% of the total connections, where only 4.49% connections were positive. With respect to heavy metals, they exhibited more connections with ARGs, which significantly occupied 46.1% of the total connections. Among the connections, 28.1% were positive, while 18.0% were negative. These experimental results revealed that the environmental transport behavior of TCs and heavy metals was

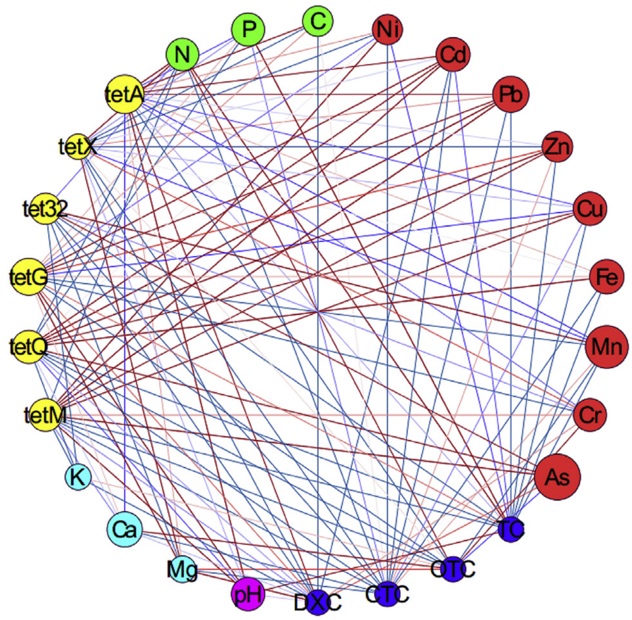

Fig. 7. Network analysis on the occurrence of TCs、ARGs and other compositions in the recovered solids. Blue nodes represented TCs, and yellow, red, green, light blue, pink ones were ARGs, heavy metals, other elements, metals, reaction parameters, respectively. Each connection with red line represented a positive and significant correlation ($P < 0.05$). Each connection with blue line represented a negative and significant correlation ($P < 0.05$). Edge weigh was calculated according to the correlation coefficient. The size of each node is proportional to the number of connections. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

inconsistent with ARGs, which were also confirmed by other studies (Mckinney et al., 2010; Zhao et al., 2019). However, different from the mechanism of selective pressure (Wang et al., 2019a; Zhao et al., 2019), the effects of antibiotic and heavy metals on ARGs migration in the present study were ascribed to the physiochemical process of struvite crystallization. It has been reported that the nucleic acid molecule of DNA possessed several functional group, like ribose, pyrimidine amino and purine, which could easily chelate with heavy metals, organics, precipitates, through hydrogen boding, electrostatic interaction and cation bridging et al. (Pils and Laird, 2007; Sheng et al., 2019; Wang et al., 2018a; Zhai et al., 2019). Under struvite crystallization, several mechanisms might occurred simultaneously, including the complexation of heavy metals with ARGs to form chelates (Hou et al., 2014; Sheng et al., 2019; Wang et al., 2018a), TCs and ARGs simultaneously combining with organics (Ye et al., 2020; Zhai et al., 2019), TCs and ARGs adsorption by struvite crystals (Ye et al., 2017; Yee et al., 2019), co-precipitation of chelates of heavy metal, TCs and ARGs compounds, etc. Such complex and co-existed processes need more stringently conditional experiments to investigate in the near future.

As described in the above text, Mg, K, Ca, NH_4—N, P and C could be used to represent the components in the recovered solids, where their content were listed in Table 1. The combined effects of Mg, K, Ca, NH_4—N and P occupied 22.5% of the total connections, in which 7.89% had positive effects and 14.6% negative effects. It has been reported that struvite and K-struvite shared the same crystal structures, where they contained PO_4^{3-}, NH_4^+ or K^+ and octahedral of $Mg[H_2O]_6^{2+}$, and the rectangular *ac* or *bc* crystallite facets were rich in $Mg[H_2O]_6^{2+}$ with positive charges (Prywer and Torzewska, 2009; Ye et al., 2014). Since ARGs were deprotonated and negatively charged under alkaline conditions (Izatt et al., 1970; Liu, 2012), they were prone to be adsorbed by $Mg[H_2O]_6^{2+}$ in struvite crystallite facets. Therefore, 3.98×10^4—5.66×10^7 copies/g of ARGs adsorption by struvite particles were observed in synthetic wastewater, as displayed in Fig. 4. With respect to the negative effects of compositions, previous researches revealed that ionic and cationic ions, including Mg^{2+}, Ca^{2+}, NH_4^+ and phosphate (Romanowski et al., 1991; Wang et al., 2018a; Zhai et al., 2019), could easily chelate with ARGs to form stable compounds, which might resist ARGs migration from the liquid to the recovered solids. Organic matters (element C) displayed 3.37% significance on the total connections, with 2.25% positive and 1.12% negative effects. According to the literature, ARGs could complex with various kinds of organics through different mechanisms (Xia et al., 2019; Zhai et al., 2019), which might boost or hinder ARGs transport from the wastewater to the final recovered products coexistently.

pH was adopted to evaluate the effects of struvite precipitation under alkaline circumstance. As shown in Fig. 7, pH possessed 3.37% positive and 2.24% negative effects on the total connections. The positive effects suggested that struvite recovery boost ARGs migration, which has been illustrated by the present study. With regard to the negative effects, pH at 9.5 for struvite precipitation might increase the hydrophilia of ARGs, since the deprotonation of thymine and guanine bases of DNA occurred at pH above 9.0 (Liu, 2012). In this regard, it would be harder to drag ARGs from the liquid phase to the solid phase.

5. Conclusion

This study was conducted to investigate the simultaneous residue of TCs and ARGs in struvite recovery from real swine wastewater, and discrepant factors affecting the migration of TCs and ARGs were also screened out. Results revealed that TCs and ARGs were significantly detected in the recovered solids. The correlational relationship revealed that predominant factors affecting TCs and ARGs transports from the wastewater to the final products were different. Results from the redundancy analysis revealed that mineral components in the recovered products, including struvite, K-struvite and amorphous calcium phosphate, coupling with organic contents, displayed insignificant roles on TCs residues, where heavy metals posed positive and remarkable functions to boost TCs migration. In addition, the network analysis was conducted to explore the factors determining ARGs transport. The links connecting TCs and ARGs accounted for 22.5% of the total connections with 4.49% positive connections, indicating that negative correlations were predominated between ARGs and TCs. Heavy metals displayed 28.1% positive and 18.0% negative consequences on ARGs residues, suggesting that various heavy metals exerted inconsistent effects. Mineral compositions and pH possessed mixed functions on ARGs transport. Future works will be extended to investigate the effects of specific parameters on the migration of TCs and ARGs in the process of phosphorus recovery, and the adsorption mechanisms will be discussed.

Author contribution

Jiasheng Cai: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Zhi-Long Ye: Conceptualization, Methodology, Formal analysis, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition, Chengsong Ye: Methodology, Resources, Xin Ye: Methodology, Shaohua Chen: Resources

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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