Removal process of antibiotics during anaerobic treatment of swine wastewater

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Abstract

High concentrations of antibiotics in swine wastewater pose potentially serious risks to the environment, human and animal health. Identifying the mechanism for removing antibiotics during the anaerobic treatment of swine wastewater is essential for reducing the serious damage they do to the environment. In this study, batch experiments were conducted to investigate the biosorption and biodegradation of tetracycline and sulfonamide antibiotics (TCs and SMs) in anaerobic processes. Results indicated that the removal of TCs in the anaerobic reactor contributed to biosorption, while biodegradation was responsible for the SMs' removal. The adsorption of TCs fitted well with the pseudo-second kinetic mode and the Freundlich isotherm, which suggested a heterogeneous chemisorption process. Cometabolism was the main mechanism for the biodegradation of SMs and the process fitted well with the first-order kinetic model. Microbial activity in the anaerobic sludge might be curtailed due to the presence of high concentrations of SMs.

Keywords: Antibiotics, anaerobic treatment, swine wastewater, biosorption, biodegradation

1. Introduction

Conventional small-scale swine husbandry has in recent decades been transformed into an intensive swine industry due to people's increasing demand for meat (Feng et al., 2017). To maintain the swine health and limit disease transmission and ensure that pigs can be kept in a high-density and closed system, veterinary antibiotics are widely used in swine farms to treat and prevent diseases (Sarmah et al., 2006). Moreover, antibiotics are usually used as feed additives to improve the growth rate and efficiency of pigs. However, antibiotics are poorly absorbed by pig guts, and around 70%-90% of them are excreted via urine and faeces based on the used antibiotics' compounds (Cheng et al., 2018b). Tetracycline antibiotics (TCs) and sulfonamide antibiotics (SMs) are the most widely used antibiotics on swine farms

due to their low costs and broad range of activity (Hruska & Franek, 2012; Koike et al., 2007). As reviewed by Cheng et al. (2018b), TCs and SMs have been frequently detected in swine wastewater at concentrations of up to 316.5 μg/L and 685.6 μg/L, respectively. Therefore, swine wastewater is a significant source for the spread of TCs and SMs into the environment.

The increasing presence of antibiotics in the environment could cause adverse outcomes for people's health and ecological safety, which has become a major concern worldwide (Richardson & Ternes, 2005). Reports by previous researchers have stated that antibiotics can affect the composition, growth, respiration and enzyme activity of aquatic and terrestrial microorganisms (Brandt et al., 2015; Välitalo et al., 2017; Zhou et al., 2013). In addition, long-term exposure of antibiotics will generate antibiotic-resistant bacteria and antibiotic-resistant genes (ARGs), which have been considered as emerging contaminants (Zhang et al., 2009). In the water environment, ARGs can easily transfer to both human and animal pathogens through horizontal gene transfer, creating a severe health risk to humans and animals by greatly limiting the efficacy of antibiotics that have been developed to treat infectious diseases (Ma et al., 2018). For this reason, removing antibiotics from swine wastewater is now critical if the adverse effects of antibiotics and ARGs on the environment and human health are to be mitigated.

The anaerobic treatment process is one of the mostly widely used technologies for high-strength swine wastewater, considering it is characterized by low power consumption and high energy recovery potential (Sakar et al., 2009). Although anaerobic treatment processes have been considered able to remove antibiotics to various extents, based on their type and concentration as well as operating conditions of the process, most prior studies mainly considered the removal of antibiotics under aerobic conditions (Cheng et al., 2018b). Moreover, studies about the removal of antibiotics in anaerobic wastewater treatment

processes only focused on their removal efficiency, while information about the removal mechanisms of different classes of antibiotics is still limited. Biosorption and biodegradation have been suggested as the two main mechanisms influencing the removal of antibiotics during biological wastewater treatment processes (Cheng et al., 2018b; Li & Zhang, 2010). Considering the further reuse of the effluent and waste sludge from anaerobic treatment processes of swine wastewater, it is essential to move from merely monitoring the removal efficiencies to understanding the bioadsorption and biodegradation of antibiotics during anaerobic treatment processes. Therefore, the objective of the present study was to: 1) investigate the fate of TCs and SMs during anaerobic treatment processes; and 2) determine the biosorption and biodegradation mechanism of selected antibiotics.

2. Materials and methods

2.1 Materials

Target antibiotics, specifically tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), sulfamethoxazole (SMX), sulfamethazine (SMZ) and sulfadiazine (SDZ) were purchased from Sigma-Aldrich, Australia. LC-MS grade acetonitrile and methanol used for sample preparation and liquid chromatography analysis were also obtained from Sigma-Aldrich, Australia. Stock solutions of TCs and SMs (1000 mg/L) were prepared by dissolving each compound in methanol, and stored at −15 °C in a refrigerator before use. The experimental solution was obtained by diluting the stock solution into the required concentrations.

Then the synthetic wastewater was prepared based on the characteristics of swine wastewater reported recently (Xu et al., 2019). The composition of the synthetic swine wastewater used in this study mainly contained: 3000 ± 200 mg/L of COD (supplied by glucose), 223 ± 45 mg/L of NH_4Cl, 65.8 ± 15 mg/L of KH_2PO_4, 54 ± 5 mg/L of

$MgSO_4 \cdot 7H_2O$, and 4 ± 0.05 mg/L of $CaCl_2 \cdot 2H_2O$. A stock solution of sythetic swine wastewater was prepared at relatively high concentration and stored in fridge at 4.5 ℃ for 5 days. For each experiment, the stock solution was diluted by distilled water to the required concentration. Anaerobic sludge employed in this study was collected from the Cronulla wastewater treatment plant in New South Wales, Australia, and acclimated in an upflow anaerobic sludge blanket reactor with synthetic swine wastewater without the addition of antibiotics.

2.2 Experimental setup and operating conditions

Firstly, two series of batch experiments were conducted in 150 ml glass bottles with non-sterile and sterile sludge (0.15 g NaN_3 was added into each bottle to inhibit the activity of anaerobic microorganisms), in order to examine the removal fate of antibiotics in contact with anaerobic sludge. The design of this experiment is summarized in Table 1.

Table 1

The selected two classes of antibiotics (TCs and SMs) were spiked into the bottle separately with the initial concentrations of 300 μg/L and 100 μg/L, respectively. Following this, the glass bottles were completely sealed with rubber plugs and N_2 was sparged for 2 min in each bottle to displace any oxygen present. The bottles were shaken on a thermostatic rotary shaker at 125 rpm and at room temperature (~25 °C). The mixed liquor suspended solids (MLSS) concentration was around 5000 mg/L in the reactor and pH=7.5 ± 0.1. Based on experimental results derived from the first stage, the experiment on the biosorption and biodegradation mechanisms of antibiotics would be conducted in the following step. Control experiments, TCs and SMs solution without the addition of anaerobic sludge, and anaerobic sludge without TCs and SMs were conducted to avoid their photodegration/adsorption on containers and their residue in the sludge. All experiments were conducted in duplicate. The sample (2 ml) collected from the bottle at each sampling time was centrifuged at a speed of

3500 rpm for 5 min. The supernatant was then filtered through a syringe filter (0.2 μm) before LC-MS/MS analysis.

2.3 Analytical methods

The chemical oxygen demand (COD) concentration was measured according to the Standard Methods by using the test kit designated HI93754B-25 (Hanna Instruments Australia, Melbourne, Australia) (Federation & Association, 2005). Mixed liquor suspended solids (MLSS) was measured according to the standard method by filtering the mixed sludge with 0.7 μm glass fiber followed by drying at 105 °C. The concentration of antibiotics was determined by Shimadzu LCMS-8060 triple quadrupole mass spectrometer. A Phenomenex C18 column (Luna, 3.0 × 100 mm, 3 μm) was used at a constant temperature of 28°C to separate the antibiotics. Water and acetonitrile with 0.1% (V/V) formic acid served as mobile phase A and mobile phase B, respectively. The LC gradient started with 30% of mobile phase B, which was retained for 7 min. Thereafter, the concentration of B increased to 95% and held for an equilibration time of 3 min. It was returned back to 30% over 3 min until the next injection. The flow rate was 0.4 mL/min, and the injection volume was 1 μL. Electrospray positive ion mode (ESI+) was used for the mass spectrometry operation. The multiple reaction monitoring (MRM) mode with two mass transitions was selected for the quantitation. The interface voltage was set at 4.0 kV. The nebulizing gas and heating gas were using a flow rate of 3.0 and 10.0 L/min, respectively. The interface temperature was held at 300 ℃.

3. Results and discussion

3.1 Removal of TCs and SMs in anaerobic sludge reactors

The concentration variation of the selected antibiotics in the anaerobic reactor during the 120 h experimental period is presented in Fig. 1 (a), which reveals that a similar removal trend and efficiency was found for TCs in the anaerobic reactor with non-sterilized and

sterilized sludge. All of them can be rapidly and significantly removed in both reactors (R1 and R2). Thus, the major removal route for TCs in the anaerobic reactor was adsorption rather than biodegradation. TCs were adsorbed onto the anaerobic sludge immediately after they made contact with the anaerobic sludge (>90% in the first 30 min), possibly due to the abundance of active sites on the adsorbent's surface. The rapid and strong adsorption of TCs onto solid matter during anaerobic digestion of animal manure or aerobic sludge has been reported by other researchers (Álvarez et al., 2010). For instance, Huang et al. (2012), Prado et al. (2009) and Kim et al. (2005) found no biodegradation of TCs in the aerobic activated sludge system, and sorption contributed to be the principal removal mechanism. As shown in Fig. 1 (a) and (c), the removal mechanism and efficiency of TC, CTC and OTC were quite similar in the anaerobic reactor, although they belong to different subclasses of TCs.

Figure 1

The concentration of SMs in the reactor with the activated sludge deceased gradually while the change in the concentration was almost negligible in the reactor with the sterilized sludge (Fig. 1(b)). This outcome reflected the fact that the continual reduction of SMs is attributed to biodegradation by anaerobic microorganisms. Similar results have been found for the biodegradation of SMs in aerobic sludge processes in other studies (Yang et al., 2011; Yang et al., 2012). They explained that SMs with low n-octanolewater distribution coefficients (log Kow) have high water solubility and their adsorption onto activated sludge was negligible. According to the pK_{a1} and pK_{a2} values of SMs (1.85, 5.6 for SMX, 2.07, 7.65 for SMZ, 1.57, 6.5 for SDZ, respectively), the predominant species of SMs would be in the form of anion at the study pH of 7.5. Thus, SMs adsorb less due to electrostatic repulsion by the negatively charged surface of the anaerobic sludge (Cheng et al., 2018b; Oberoi et al., 2019). Similar to SMs, TCs also have low log K_{ow} and high water solubility, so that their adsorption onto the activated sludge was not caused by hydrophobic interactions. Conversely,

based on the pK_a values of TCs (3.32, 7.78, 9.58 for TC, 3.22, 7.46, 8.94 for OTC, and 3.33, 7.55, 9.33 for CTC, respectively), they could exist in a neutral form at pH 7.5 that was more amenable to adsorptive removal via electrostatic interactions between the zwitterionic species and negatively charged surface of biological sludge (Oberoi et al., 2019, Wang et al., 2015)

3.2 Adsorption process of TCs onto anaerobic sludge

To investigate the adsorption process of TCs onto anaerobic sludge, a series of batch adsorption experiments were conducted in 150 mL glass bottles with 100 mL sterile sludge to avoid the biodegradation of TCs. The glass bottles were shaken in an orbital shaker at 125 rpm. An experiment for the kinetics study was done by using 300 μg/L of TCs adsorbed onto different amounts of anaerobic sludge (MLSS = 1000, 2000 and 3000 mg/L). The variation in adsorption capacity of anaerobic sludge at different times is presented in Fig. 2. For isotherm studies, experiments with varying initial concentrations (50, 100, 200, 300, and 500 μg/L) were conducted. Control experiments at the same initial TCs concentration without the addition of sludge were also prepared under the same laboratory conditions and no significant loss was documented. Based on the results of the kinetics experiments, the biosorption of TCs on anaerobic sludge could reach equilibrium within 12 h, the aqueous TCs' concentrations changed very little once adsorption equilibrium had been achieved.

Figure 2

In the present study, pseudo-first-order and pseudo-second-order equations were separately used for the regression of the adsorption process of TCs onto anaerobic sludge. The experimental results are summarized in Fig. 2 and Table 2, which fit well to the pseudo-second-order equation with higher correlation coefficients than the pseudo-first-order model. Meanwhile, the theoretical values of q_e calculated from the pseudo-second-order model correspond well with the experimental q_e values. Thus, the pseudo-second-order model is more suitable to describe the behavior of the adsorption process than the pseudo-first-order

kinetic model. This is consistent with the results of previous studies that investigated the adsorption of TCs onto anaerobic and aerobic sludge (Huang et al., 2012; Li et al., 2013). Such results suggested that: firstly, chemisorption may be the rate-limiting step; and secondly, the sorption capacity was proportional to the number of available active sites on the sorbent. The process involves exchange or sharing of electrons mainly between cation and functional groups (hydroxyl and carboxyl groups) of the biomass cell (Michalak et al., 2013). Moreover, the increase of the pseudo-second-order rate constant (k_2) was observed when the sludge concentration changed from 1000 mg/L to 3000 mg/L, which might due to the available adsorption sites increased with increasing amount of adsorbent. However, the equilibrium adsorption capacity of anaerobic sludge decreased when the initial sludge concentrations at the same initial concentration of TCs were increased (Fig. 2). A possible explanation for this is that the increase in adsorption sites resulted in unsaturated adsorption surfaces at a constant amount of TCs (Mihciokur & Oguz, 2016).

Table 2

Langmuir and Freundlich isotherm models were used to evaluate the adsorption data of TCs onto anaerobic sludge. The Langmuir equation assumes that the adsorption covers the homogeneous surface of adsorbent and the adsorbate molecules are non-interactive, while the Freundlich isotherm is suitable for adsorption on a heterogeneous surface, which assumes that the adsorption occurs at available sites on the surface with a different free energy (Ayawei et al., 2017). As shown in Table 2 and Fig. 3, the Freundlich model with a larger correlation coefficient (R^2= 0.976 - 0.993) fits better to the experimental data than the Langmuir model (R^2=0.945-0.968), suggesting that the adsorption of TCs onto the anaerobic sludge is a complex heterogeneous surface adsorption. The heterogeneous structure of extracellular polymeric substances (EPS) produced by activated sludge may affect the adsorption process (Song et al., 2014). As well, the 1/n values obtained by the Freundlich

model are lower than 1.0, which means that TCs' adsorption on anaerobic sludge is a favorable process (Ahmed, 2017).

Figure 3

3.3 Degradation of SMs in anaerobic sludge

As shown in Fig. 1 (b), the removal of SMs in the anaerobic sludge is due to the role of biodegradation. The experiment on the biodegradation kinetics of SMs was also explored in batch experiments. The initial SMs concentrations were 100, 200 and 300 μg/L, respectively, under the pH of 7.5 ± 0.1 and at room temperature for 120 h. According to the removal efficiency vs. time profiles (shown in Fig. 4(a)), the concentration of SMX, SDZ and SMZ decreased steadily in the first 72 h of the experiment, with the removal efficiencies being 84.2-91.0%, 5.5-21.1% and 18.3-25.3% in 72 h, respectively. During this experimental period, the degradation ratio of SMs in anaerobic sludge was in the order of SMX>SMZ>SDZ, with the values of 97.4-98.9%, 12.0-31.2% and 23.9-33.5%, respectively.

The biodegradation data of SMX, SDZ and SMZ in anaerobic sludge were analysed by using the first-order kinetic model, as shown in the following kinetic formula:

$$\frac{dC}{dt} = -k_1 \cdot C \leftrightarrow C_t = C_0 \cdot e^{-k_1 \cdot t}$$

Where, C_0 is initial concentration of the antibiotic added in the sludge; C_t is concentration of the antibiotic at time t; and k is the degradation rate constant. Using this equation, half-lives, $t_{1/2}$ can be calculated as (DT50=ln 2/k).

The degradation of SMX, SDZ and SMZ in anaerobic sludge fitted well with the first-order reaction kinetic model, with all R^2 values ranging from 0.84 – 0.99, as presented in Fig. 4 and Table 3.

Table 3

Comparatively, the degradation rate of SMX appeared to be much faster than that of SDZ and SMZ, of which more than 50% can be degraded in less than 23 h. SDZ and SMZ showed a

persistent ability to be degraded in anaerobic sludge with the DT50 values of 223.6-577.6 h and 203.9-346.6 h, respectively, with the initial concentration of 100-300 μg/L. The fast and large removal of SMX also has been detected in previous research studies. For example, Feng et al. (2017) and Mohring et al. (2009) concluded that the SMX in swine manure was almost 100% degraded and rapidly. Larcher and Yargeau (2012) also indicated that the removal rate of SMX could achieve > 99% with both low (4 to 400 μg/L) and high (2 to 10 μ) initial SMX concentrations. Feng et al. (2017) found no biodegradation for SDZ during anaerobic digestion of swine manure. The persistence of SMZ during anaerobic fermentation was also found by Mohring et al. (2009), who discovered that 100% of the initially measured concentration of sulfamethazine (SMZ) could still be detected after a 34-day fermentation period. The different functional group of SMX, SMZ and SDZ may contribute to their varying degradation rates. Yang et al. (2016) explained that functional groups may contribute electronegativity effects that inhibit the degradation of SMZ and SDZ by influencing their interaction with the microbes.

The initial concentration of antibiotics wielded some effects on the degradation rate of SMs, and the degradation would be slower at a higher exposure level (Shen et al., 2018). In the present study, all of these three antibiotics with the initial concentration of 100 μg/L revealed the lowest DT50 values, whereas higher concentrations of antibiotics (200 μg/L) caused a lower degradation rate and longer persistence. The review paper by Cheng et al. (2018a) indicated that higher dosages of antibiotics showed more inhibition of microbial activity which in turn inhibited the degradation of SMs. Yang et al. (2016) also suggested that degradation kinetics of SMs depended on the initial concentrations and removal rates would be slower at a higher concentration. However, the removal efficiency and degradation rate of SMs only indicated a slight change when increasing the concentration from 200 to 300 μg/L, which means the microbial community in anaerobic sludge could adapt to the presence of

SMs. As well, microorganisms in anaerobic sludge that are able to degrade SMs might be enriched by increasing the concentration of SMs (Cycoń et al., 2019).

Co-metabolism is regarded as an important mechanism for the biodegradation of antibiotics in the biological wastewater treatment process (Cheng et al., 2018b; Oliveira et al., 2016). In this study, batch experiments were conducted with different initial concentrations of COD to investigate the effect of COD on the biodegradation of SMs in anaerobic reactors. Different COD concentrations in the reactor were obtained by diluting the stock solution of synthetic swine wastewater to 1500, 1800, 2700 and 4500 mg/L, respectively. The experiment was run under the same conditions with the above experiment by using 100 μg/L of SMX, SMZ and SDZ, respectively. As shown in Fig. 5, the enhanced removal of SMs has been observed by increasing the COD concentration from 1500 to 2700 mg/L. By the end of 120 hours reaction, the efficiencies in removing SMX, SMZ and SDZ rose, respectively, from 76.06% to 98.69%, 10.58% to 32.53%, and 11.17% to 30.65%. Thus, an increasing trend was observed for the biodegradation of SMs in the anaerobic reactor by increasing COD concentrations, although a slight decline was found when the COD concentration further increased to 4500 mg/L. This finding indicated that the presence of easily biodegradable substrates, such as glucose used in this study, could enhance the biodegradation of SMs, which suggested the degradation mechanism of cometabolism. What was observed in this study agrees with previous recent analyses by Oliveira et al. (2016) and Oliveira et al. (2019), who demonstrated that the addition of readily available organic matter enhanced the removal efficiency of SMZ in anaerobic treatment processes.

Figure 5

Additionally, a clear correlation between COD consumption and SMs removal can be observed from Fig. 4 (a). The removal rate of SMs is positively correlated with the consumption of COD, and more COD was consumed when achieving higher removal

efficiencies of SMs. Oliveira et al. (2017) and Alvarino et al. (2014) also observed a linear relationship between COD removal rate and the biodegradation rate of SMs during anaerobic processes. These results also reflected the cometabolic biodegradation of SMs in anaerobic processes. As displayed in Fig 4 (a), the removal efficiency of COD fell from 58.76% to 51.65% by increasing the initial concentration of SMs from 100 to 200 μg/L, which dropped to only 18.82% when 300 μg/L of SMs was added to the reactor. The degradation of organic pollutants in anaerobic treatment processes is reliant on the synergistic cooperation of various microbial groups forming a metabolic network (Stams, 1994). Thus, the decline in the COD removal efficiency may result from the inhibition effect of SMs on microbial activity under higher concentrations. However, the removal efficiency of SMs was not limited by raising their initial concentrations from 200 to 300 μg/L. This finding indicated that the cometabolic biodegradation of SMs was determined specifically by cometabolism instead of the overall metabolism, which was caused by specific groups of microorganisms. Similar results were concluded by Barret et al. (2010), and these authors demonstrated that the cometabolic biodegradation of pharmaceutical compounds would be mainly affiliated with specific metabolic stages of the whole biodegradation process. Oliveira et al. (2017) also indicated that the micropollutant transformation is expected to be associated with particular metabolic pathways. This is consistent with a cometabolic transformation caused by the non-specificity of specific enzymes that occasionally convert the micropollutant along with its main substrate.

4. Conclusions

This study investigated the removal mechanism of TCs and SMs in anaerobic sludge and found that TCs were removed through adsorption of anaerobic sludge while SMs were eliminated through biodegradation. The adsorption of TCs onto the anaerobic sludge fitted

well with the pseudo-second kinetic mode and the Freundlich isotherm, suggesting the importance of a heterogeneous chemisorption process. The degradation of SMs in anaerobic processes fitted well to the first-order kinetic model. SMX was the most easily biodegradable antibiotic with the lowest DT50 values. The degradation of SMs occurred via the cometabolism triggered by specific microbial communities.

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Figure Captions

Fig. 1 The concentration variation of tetracycline antibiotics (a) and sulfonamide antibiotics (b); and their removal efficiency (c) in the reactor with non-sterile and sterile anaerobic sludge.

Fig. 2 Adsorption kinetics data and fitted modes of tetracycline (TC) (a), chlortetracycline (CTC) (b) and oxytetracycline (OTC) (c) onto different concentrations of anaerobic sludge

Fig. 3 The adsorption isotherms of tetracycline (TC), chlortetracycline (CTC), and oxytetracycline (OTC) onto anaerobic sludge.

Fig. 4 (a) Removal efficiencies of sulfonamide antibiotics and COD in the anaerobic reactor; First-order biodegradation kinetic model of sulfamethoxazole (SMX) (b), sulfadiazine (SDZ) (c) and sulfamethazine (SMZ) (d).

Fig. 5 Removal efficiencies of sulfamethoxazole (SMX), sulfadiazine (SDZ) and sulfamethazine (SMZ) in the anaerobic reactor with different concentrations of COD.

Table Captions

Table 1 Experimental design of the batch tests

Table 2 Kinetic and isotherm models and parameters for the adsorption of tetracycline antibiotics onto anaerobic sludge

Table 3 Degradation rate constants (k1) and half-lives (t1/2) of the three sulfonamide antibiotics in anaerobic reactor

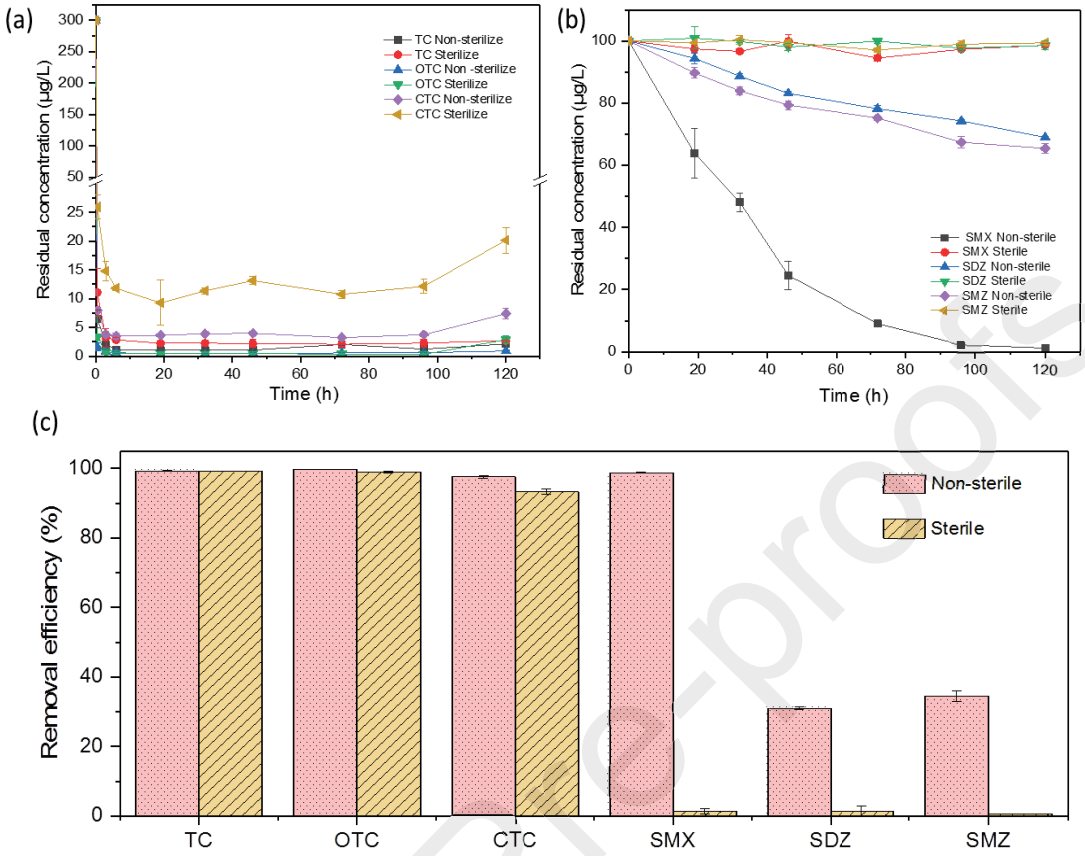

Fig. 1 The concentration variation of TCs (a) and SMs (b); and their removal efficiency (c) in the reactor with non-sterile and sterile anaerobic sludge.

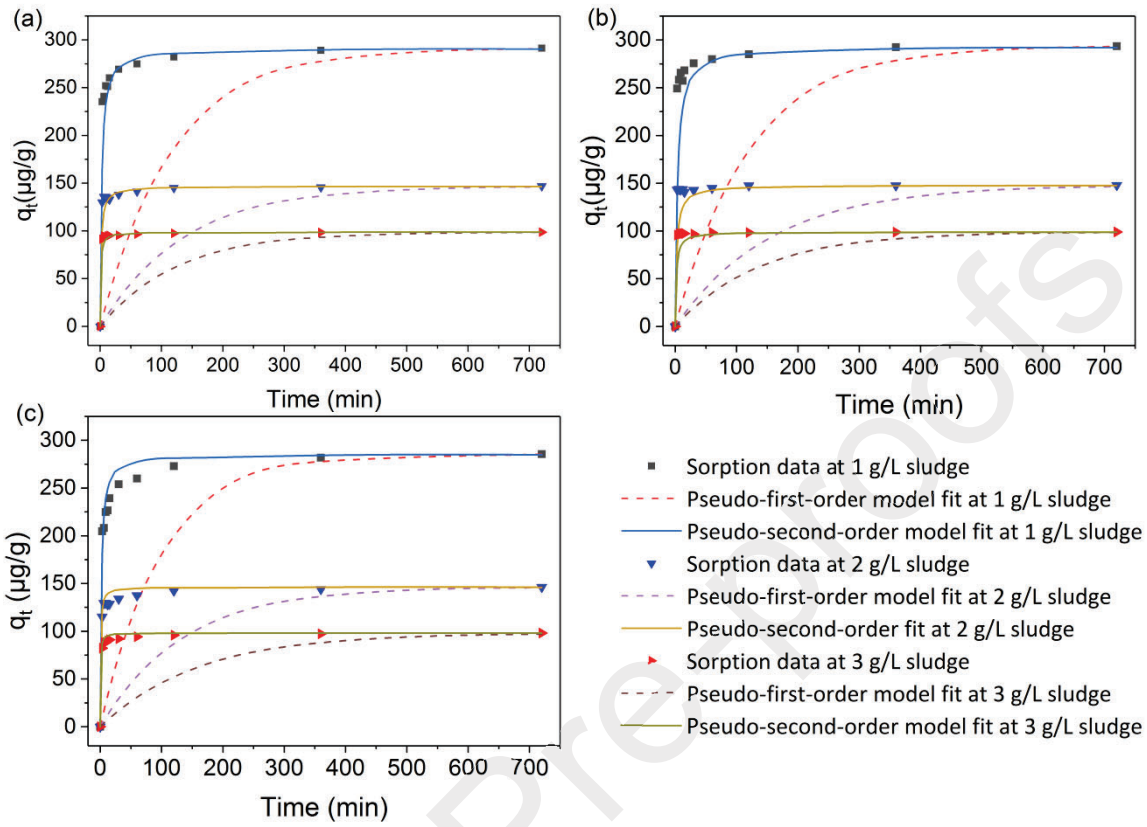

Fig. 2 Adsorption kinetics data and fitted modes of tetracycline (TC) (a), chlortetracycline (CTC) (b) and oxytetracycline (OTC) (c) onto different concentrations of anaerobic sludge

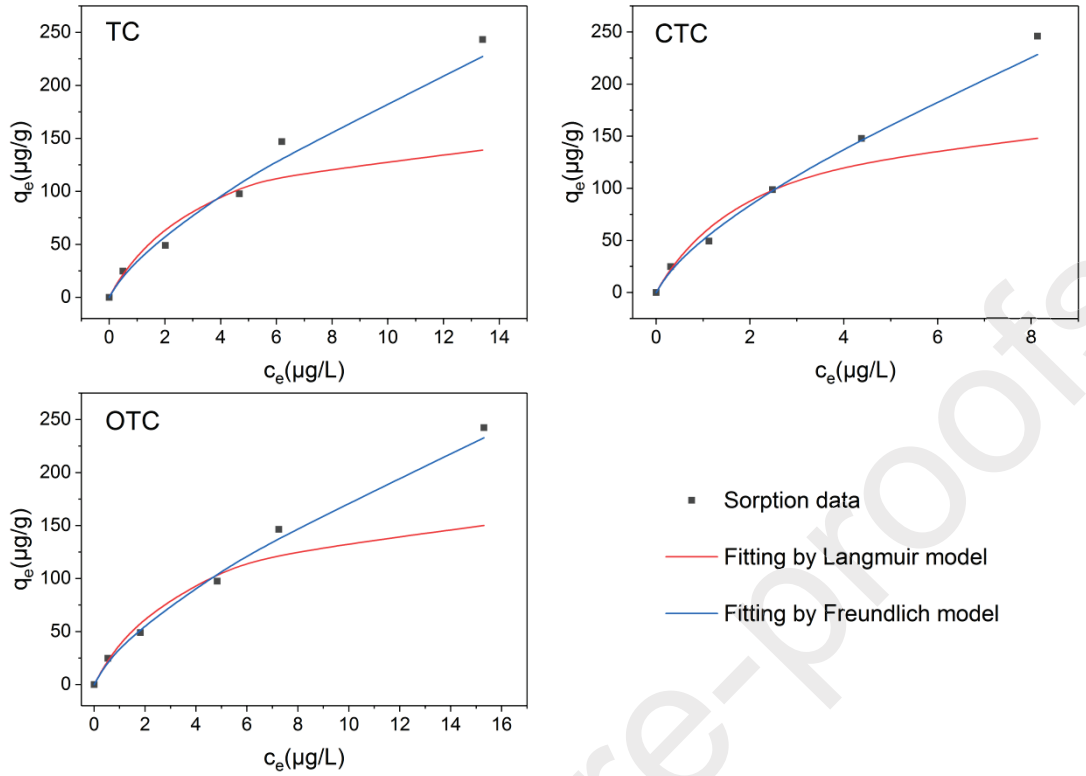

Fig. 3 The adsorption isotherms of tetracycline (TC), chlortetracycline (CTC), and oxytetracycline (OTC) onto anaerobic sludge.

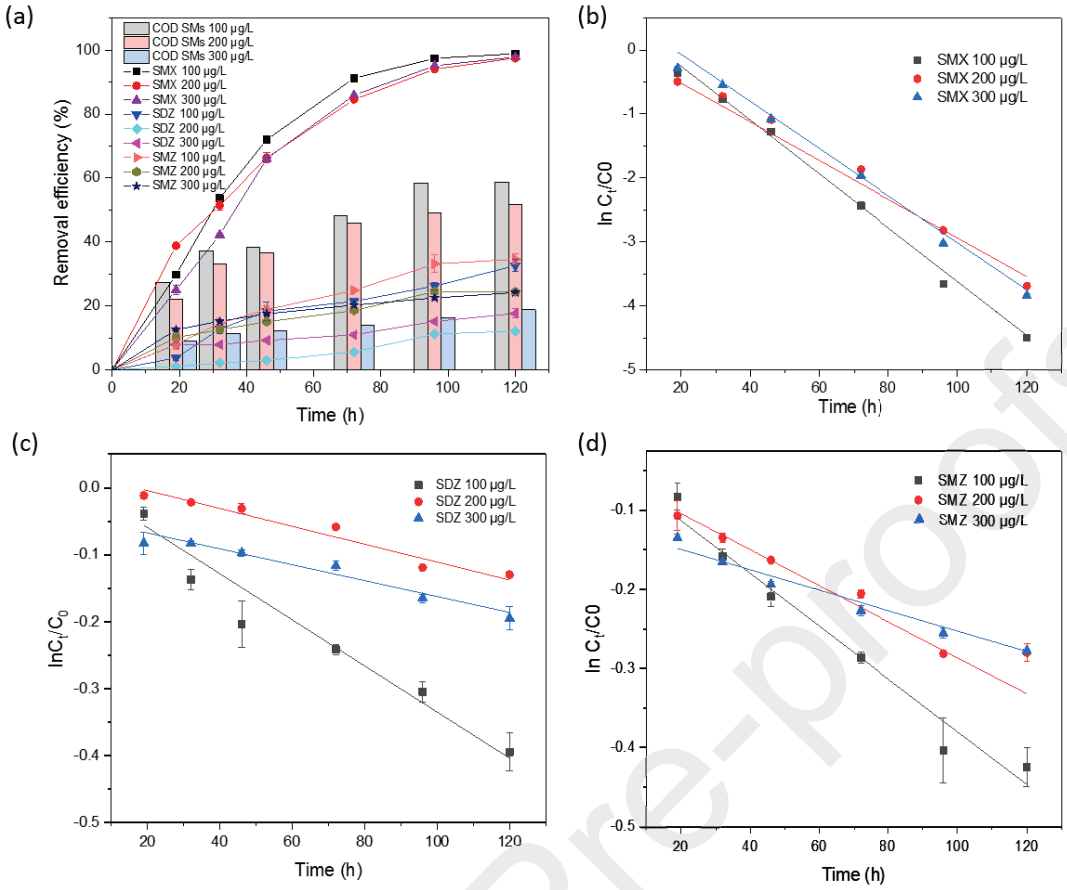

Fig. 4 (a) Removal efficiencies of sulfonamide antibiotics and COD in the anaerobic reactor; First-order biodegradation kinetic model of sulfamethoxazole (SMX) (b), sulfadiazine (SDZ) (c) and sulfamethazine (SMZ) (d).

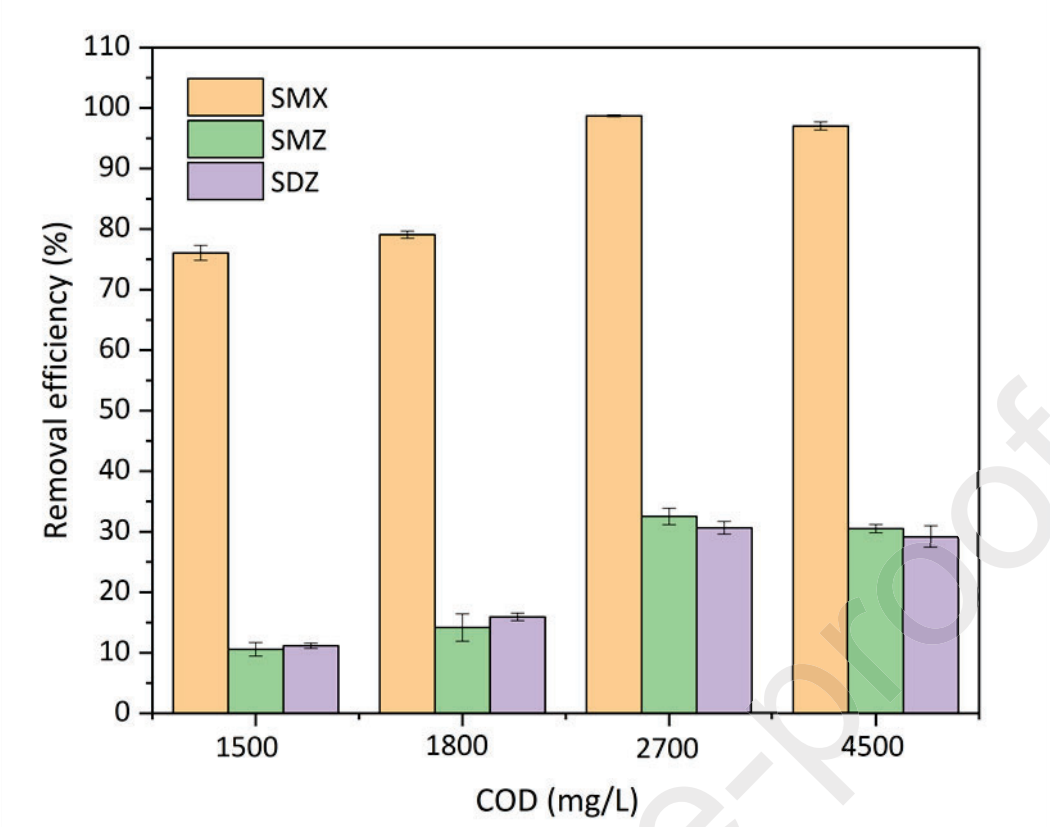

Fig. 5 Removal efficiencies of sulfamethoxazole (SMX), sulfadiazine (SDZ) and sulfamethazine (SMZ) in the anaerobic reactor with different concentrations of COD.

Table 1 Experimental design of the batch tests

Reactor	Anaerobic sludge	Wastewater	Antibiotics	NaN_3
R1/R1'	+	+	TCs/SMs	-
R2/R2'	+	+	TCs/SMs	+
R3/R3'	-	+	TCs/SMs	+
R4	+	+	-	-

"R (1,2,3)" and "R'(1,2,3)" represented the reactor with the addition of TCs and SMs, respectively. "+" indicated "with", "-" indicated "without".

Table 2. Kinetic and isotherm models and parameters for the adsorption of tetracycline antibiotics onto anaerobic sludge

Model	Equation	Parameter	TCs		
			TC	OTC	CTC
Pseudo-first-order kinetics	$ln\,(q_e - q_t) = lnq_e - k_1 t$	q_e	38.59 [a], 11.32 [b], 4.51 [c]	32.27 [a], 4.57 [b], 1.88 [c]	56.89 [a], 17.34 [b], 9.04 [c]
		k_1	0.0085 [a], 0.0074 [b], 0.0081 [c]	0.01 [a], 0.0075 [b], 0.0063 [c]	0.0082 [a], 0.0064 [b], 0.0073 [c]
		R^2	0.9138 [a], 0.8063 [b], 0.9294 [c]	0.9644 [a], 0.6324 [b], 0.8069 [c]	0.8959 [a], 0.8015 [b], 0.8688 [c]
Pseudo-second-order kinetics	$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t$	q_e	285.5 [a], 147.06 [b], 99.01 [c]	294.12 [a], 147.06 [b], 99.01 [c]	285.71 [a], 147.06 [b], 98.04 [c]
		k_2	0.0015 [a], 0.005 [b], 0.0132 [c]	0.002 [a], 0.0113 [b], 0.0276 [c]	0.001 [a], 0.003 [b], 0.0064 [c]
		R^2	1.0 [a], 1.0 [b], 1.0 [c]	1.0 [a], 1.0 [b], 1.0 [c]	0.9999 [a], 0.9999 [b], 1.0 [c]
Langmuir	$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{K_L q_m c_e}$	K_L	0.34	0.49	0.28
		q_m	169.49	185.19	185.19

		R^2	0.9446	0.9598	0.9676
		K_F	36.3	52.34	35.47
Freundlich	$lnqe = ln\,K_F + \frac{1}{n} ln\,c_e$	1/n	0.707	0.703	0.690
		R^2	0.9760	0.9902	0.9933

k_1 is the rate constant of first-order adsorption (/min), k_2 is the pseudo-second-order rate constant (g/μg·min), q_e and q_t are the amounts of TCs adsorbed on anaerobic sludge at equilibrium and at time t (min), c_e is the equilibrium concentration of the TCs (μg/L) in the aqueous phase, K_L (L/mg) is the Langmuir bonding term related to the interaction energies, K_F (L/mg) is the Freundlich affinity coefficient, n is Freundlich linearity constant. a: 1000 mg/L MLSS, b: 2000 mg/L MLSS; c: 3000 mg/L MLSS.

Table 3. Degradation rate constants (k1) and half-lives (t1/2) of the three sulfonamide antibiotics in anaerobic reactor

Antibiotic	Initial concentration (μg/L)	$k_1(h^{-1})$	R^2	DT50 (h)
SMX	100	0.0397	0.9861	17.45963
	200	0.0306	0.9881	22.65187
	300	0.0337	0.983	20.56817
SDZ	100	0.0031	0.9826	223.5959
	200	0.0012	0.9352	577.6227
	300	0.0013	0.8659	533.1901
SMZ	100	0.0034	0.9695	203.8668
	200	0.0021	0.8946	330.0701
	300	0.0020	0.8357	346.5736