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Effects of Lighter Dose of Oxytetracycline on the Accumulation and Degradation of Volatile Fatty Acids in the Process of Thermophilic Anaerobic Digestion of Swine Manure

Zijing Fan, Mei Zhang, Xiaxia Chen, Zhongda Hu, Qihang Shu, Chaosen Jing and Xingzhang Luo *

Department of Environmental Science and Engineering, Fudan University, Shanghai 200433, China; 18210740041@fudan.edu.cn (Z.F.); 18210740070@fudan.edu.cn (M.Z.); 19210740030@fudan.edu.cn (X.C.); 17210740025@fudan.edu.cn (Z.H.); 19210740047@fudan.edu.cn (Q.S.); 15110740018@fudan.edu.cn (C.J.)

* Correspondence: lxz@fudan.edu.cn; Tel.: +86-18621080212

Citation: Fan, Z.; Zhang, M.; Chen, X.; Hu, Z.; Shu, Q.; Jing, C.; Luo, X. Effects of Lighter Dose of Oxytetracycline on the Accumulation and Degradation of Volatile Fatty Acids in the Process of Thermophilic Anaerobic Digestion of Swine Manure. *Sustainability* **2021**, *13*, 4014. <https://doi.org/10.3390/su13074014>

Academic Editor: Lotfi Aleya

Received: 10 March 2021

Accepted: 1 April 2021

Published: 4 April 2021

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Abstract: Oxytetracycline (OTC) is a commonly used antibiotic in livestock farming for controlling intestinal and respiratory infections in farm animals. However, the absorption of antibiotics by animals is limited, and most antibiotics are excreted in the original form with manure, which will have an impact on the environment. The removal of antibiotics from swine manure could generally be performed via anaerobic digestion (AD). In this study, the effect of oxytetracycline (OTC) at doses of 0.1, 0.5, and 1.0 mg/L on the thermophilic anaerobic digestion of swine manure (55 °C) in batch digesters was studied. The methane production, volatile fatty acid (VFA) levels, and dissolved organic matter (DOM) were determined and compared with the control (0 mg/L of OTC). The results indicate that (1) OTC at 0.1 mg/L had no inhibitory effect on methane production or on the accumulation of VFAs, while 0.5 mg/L and 1.0 mg/L inhibited methane production, with inhibition rates of 4.03% and 14.12% ($p < 0.05$), respectively; (2) the VFAs of each reactor peaked on the first day of the reaction, and as the OTC dose increased from 0 to 1.0 mg/L, the maximum VFA accumulation increased from 1346.94 mg/g to 2370 mg/g of volatile solids (VS); and (3) oxytetracycline (0.5 and 1.0 mg/L) could promote the temporary accumulation of propionic acid, which did, however, not result in significant VFA accumulation. Further, OTC at 1.0 mg/L can promote DOM production, and therefore, VFA accumulation.

Keywords: thermophilic anaerobic digestion; oxytetracycline; volatile fatty acids; methane production; swine manure

1. Introduction

Oxytetracycline (OTC) is a commonly used antibiotic in livestock farming for controlling intestinal and respiratory infections in farm animals [1]. The OTC concentrations in manure samples can be in the range of 0.006–136 mg/L [2]. However, the absorption of antibiotics by animals is limited, and more than 85% of antibiotics are excreted in their original forms with manure [3]. Antibiotic residues in the environment can affect the growth and metabolism of aquatic and terrestrial organisms, leading to antibiotic resistance in bacteria [4]. Anaerobic digestion is one of the most widely used biological treatment methods for swine farm wastewater to reduce organic solid waste via organic matter degradation and produce biogas [5]. Mesophilic temperature anaerobic digestion and thermophilic anaerobic digestion are two commonly used processes, both with their own sets of advantages and disadvantages. Although few studies have focused on thermophilic anaerobic digestion, compared with digestion at moderate temperatures, thermophilic anaerobic digestion has the advantages of a shorter residence time, a more comprehensive range of organic matter loading, and a smaller digestion tank volume. In addition, the degradation rate of organic matter is higher, resulting in the production of larger amounts of biogas [6,7]. Volatile fatty acids (VFAs), mainly composed of short-chain fatty acids

(such as formic acid, acetic acid, propionic acid, butyric acid, and valeric acid), are common products of the acidogenesis phase of anaerobic digestion, and their accumulation in the anaerobic system has an impact on biogas production capacity and organic matter degradation [8]. The presence of OTC can lead to the accumulation of VFAs and inhibit the production of methane [4,9].

However, in previous studies, the concentration range of antibiotics such as sulfonamides, tetracyclines, and macrolides was generally high (10–200 mg/L), whereas studies focusing on low OTC concentrations are scarce. In one study, low concentrations of antibiotics did not inhibit anaerobic processes [10]. However, one study has shown that tylosin inhibits the production of methane, even at concentrations as low as 0.01 mg/L [11]. Similarly, the methane production of cow manure also decreased significantly in the presence of OTC (about 3.0 mg/L) and was 52% lower than in the control digester [12]. Even at low concentrations of antibiotic mixtures (1.0 mg/L ET-ST), VFAs begin to accumulate [13]. However, there is a lack of information on the anaerobic digestion of swine manure at low OTC concentrations and high temperatures (55 °C), and to date, it is not clear whether swine manure containing low concentrations of OTC will adversely affect the gas production and antibiotic degradation efficiency during anaerobic treatment.

Researchers have different opinions on the influence of antibiotics on anaerobic reactions in the range of 0–2 mg/L. In this study, we focused specifically on the 0–1 mg/L range. In this context, we investigated the impacts of low-concentration OTC on the dynamic changes of VFAs in anaerobic fermentation at a higher temperature (55 °C) and the underlying mechanisms. First, we evaluated the effect of different OTC dosages on the production of VFAs in anaerobic fermentation. Second, we assessed the influence of OTC on anaerobic fermentation solubilization and methane production. Finally, we detected the concentration of OTC and analyzed its degradation process. Our results provide a scientific basis for considering the role of antibiotic residues in anaerobic fermentation and optimizing swine manure fermentation systems.

2. Materials and Methods

2.1. Manure Sampling and Activated Sludge

The activated sludge was obtained from the sedimentation tank of the Songjiang Qingdao Beer wastewater treatment plant in Shanghai City, China. Swine manure and rinses were obtained from a swine farm in Jiaxing, Zhejiang Province, China. The OTC was purchased from Aladdin Industrial Corporation.

2.2. Experimental Design and Procedure

Anaerobic experiments were carried out to evaluate the influence of various OTC levels in manure on thermophilic anaerobic digestion. The characteristics of the raw materials are listed in Table 1. In this study, four groups of reactors—R1, R2, R3, and R4—were established, with corresponding OTC concentrations of 0, 0.1, 0.5, and 1.0 mg/L, respectively. The schematic diagram for the experimental device is shown in Figure 1. Each reactor had a digestion liquid sampling port sealed with a rubber plug at the bottom and a gas sampling port connected to a gas collection bag at the top. Each group was subjected to anaerobic conditions (1.2 L total volume, 1 L working volume, 200 rpm stirring speed) in a water bath at 55 ± 0.5 °C for 30 days. On the basis of the sludge loading of the anaerobic digestion, 100 g of sludge and 250 g of swine manure were added to different reactors and diluted with 1 L of the rinse solution, resulting in a solid content of approximately 7% and a C/N ratio of 13.85. In the test group, 1.0, 5.0, and 10.0 mL of OTC stock solution (100 mg/L) were added, whereas the control group did not receive OTC. To maintain anaerobic conditions, prior to the experiment, the reactors were flushed with nitrogen. During the entire anaerobic process, biogas and mixed solutions were collected regularly. All samples were collected in triplicate.

Table 1. Initial substrate and inoculum characteristics.

Parameter	Unit	Inoculum Sludge	Swine Manure	Inoculum Sludge + Swine Manure + Rinse Solution
Total solids (TS)	%	22.92	23.54	7.00
Volatile solids (VS)	%	54.42	74.59	66.87
Total organic carbon (TOC)	mg/L	52.36	6901.21	4964.81
Total nitrogen (TN)	mg/L	178.04	1201.02	358.48
C/N	-	<1	5.75	13.85
pH	-	7.41	7.53	7.62

Note: TOC = total organic carbon, TN = total nitrogen, C/N = total organic carbon/total nitrogen, VS = volatile solids, and TS = total solids.

Figure 1. Schematic diagram of the experimental device.

2.3. Analytical Methods

2.3.1. Basic Indicators

Samples were taken at 1 h, 3 h, 6 h, 12 h, 24 h, 2 d, 3 d, 5 d, 8 d, 15 d, 20 d, and 30 d. The digestive juice was centrifuged at 5000 rpm for 15 min, and the clarified supernatants were used to measure the pH, total organic carbon (TOC), total nitrogen (TN), and VFAs. The pH was measured with a calibrated pH meter (AS600, Shanghai As One Trading Co., Ltd. China). The total solids (TS) and volatile solids (VS) were determined according to the American Public Health Association (APHA) standard methods [14]. The total organic carbon (TOC) and total nitrogen (TN) were tested by a TOC analyzer (TOC-L CPH, Shimadzu, Japan). The excitation emission matrix (EEM) fluorescence spectra were used to detect dissolved organic matter (DOM) via fluorescence spectrometry (Horiba, Japan).

Before measuring the VFAs, the liquid sample was diluted and filtered through a 0.45 µm filter membrane. Subsequently, 0.9 mL of the liquid was taken, and 0.1 mL of 30% phosphoric acid was added to decrease the pH to 3.0. Next, the VFAs (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid) were detected using a gas chromatograph (GC, 2010Plus, Shimadzu Corporation, Japan) equipped with a flame ionization detector (FID) and an Agilent DB-WAX UI column. The chromatographic column was first kept at 80 °C for 1 min, then heated to 180 °C at a rate of 10 °C/min and held for 5 min. The temperature of the detector and injection port were both maintained at 250 °C, with nitrogen gas flowing at 50 mL/min, hydrogen at 40 mL/min, and air at 400 mL/min as carrier gases.

2.3.2. Biogas Measurement

The biogas was collected in an air collection bag, and its volume was measured via the water displacement method. The methane in the bag was analyzed by a gas chromatograph equipped with a thermal conductivity detector (GC-960, Haixin, China) and column (Haixin ST). The carrier gas was He, and the temperatures of the injector, detector, and oven were 120, 110, and 120 °C, respectively. The cumulative biogas production during anaerobic

digestion was modeled using the modified Gompertz equation [15], which is defined as follows:

$$Y(t) = M_m \times \exp \left\{ - \exp \left[\frac{R_m \times e}{M_m} (\lambda - t) + 1 \right] \right\} \quad (1)$$

where $Y(t)$ is the cumulative methane production measurement at time t (d), M_m is the maximum cumulative methane volume (mL), R_m is the maximum methane production rate (mL/d), λ (d) is the lag time, and e equals 2.72. Additionally, these models were evaluated by statistical indicators of the coefficient of determination (R^2).

2.3.3. Ultra-Performance Liquid Chromatography–Tandem Mass Spectrometry

The OTC was extracted using the method of Dorival [16]. The ESI+ mode and internal standard method were used, and the analyzed sample injection was repeated five times. The concentration of OTC was quantified using a Xevo TQ-XS Waters LC-MS/MS with a Waters ACQUITY UPLC BEH C18 column (1.7 μ m), using the chromatographic separation of the binary mobile phase gradient, the mobile phase with a 0.1% (v/v) formic acid aqueous solution (solvent A), and methanol (solvent B). The flow rate was 0.3 L/min, the column was maintained at 30 °C, and the injection volume was 20 μ L. The mass spectrometer was a triple quadrupole mass spectrometer equipped with an ESI source and used the Masslynx software to complete instrument control as well as data acquisition and evaluation. The instrument parameters were as follows: scan time, 0.5 s; scan width, 0.2; collision gas flow (argon), 0.15 mL/min; spray voltage, 3.5 kV; cone voltage, 75 V; desolventizing gas temperature, 400 °C; desolvent gas flow rate, 800 L/hr; and cone gas flow rate, 150 L/hr.

For the most part, the degradation of organic pollutants followed a simple first-order kinetic model, whose expression is as follows:

$$dC/dt = -kC \text{ and } C_t = C_0 e^{(-kt)} \quad (2)$$

where t is time (day), C_0 and C_t are the initial concentration and concentration at time t , and k is the degradation rate constant (day^{−1}). The half-life can be expressed as follows:

$$t(1/2) = \ln 2/k \quad (3)$$

2.4. Statistical Analysis

Differences in methane contents among the control and OTC-containing serum bottles were determined by analysis of variance (ANOVA) using SPSS v. 20 (IBM, Armonk, NY, USA). Significance was accepted at probabilities (p) of 0.05 or less for all analyses. The software Origin 2020b (OriginLab Corp., Northampton, MA, USA) was used to plot the graphs.

3. Results

3.1. Effects of OTC on Methane Production

The accumulation of methane produced by anaerobic digestion at different OTC levels is shown in Figure 2. Methane production increased rapidly from 0 to 15 d in all cases, followed by a less rapid increase until the end of anaerobic digestion.

Figure 2. Daily (a) and cumulative (b) methane production for R1–R4.

On the first day, the four groups produced only low amounts of gas, most likely because in the early stage of anaerobic fermentation, microorganisms had not adapted to the wastewater environment [17]. On day 2, however, the anaerobic fermentation microorganisms started to adapt to the different conditions, and the organic matter decomposition rate increased, resulting in methane production. Groups R1, R2, R3, and R4 reached the peak of methane production at days 7, 8, 13, and 11, respectively, with values of 214.33, 205.01, 187.67, and 179.67 mL, respectively. It can be observed that the gas production peaks of R3 and R4 had a lag period. From the 20th day to the end of anaerobic digestion, the gas production of all groups began to decline. This may be due to the continuous consumption of organic matter in the raw materials, which led to biogas production continuously decreasing throughout the experiment, which has also been observed in a previous study [18].

Methane production was negatively affected by the OTC concentration. Based on Figure 2, the cumulative methane volume of the control group was slightly higher than that of the experimental groups R3 and R4 during the entire operation of the system.

The cumulative methane production levels of groups R2, R3, and R4 were 1510.10, 1400.67, and 1277.33 mL, respectively, whereas that of the control group was 1487.36 mL. The methane production of R3 and R4 decreased by 86.69 mL and 210.03 mL, respectively, and the inhibition rates were 4.03% and 14.12% ($p < 0.05$), respectively. Peak gas production was at days 7, 8, 13, and 11 for groups R1, R2, R3, and R4, respectively. Groups R3 and R4 not only had lower peak gas productions compared with the control group, but also delays in time (by 6 and 4 days, respectively). This can be explained by the fact that OTC inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit and is mainly effective for gram-negative organisms. Therefore, OTC affected the activity of microorganisms during anaerobic treatment, thereby affecting methane production [9]. Group R2 showed no inhibition of anaerobic fermentation; methane production was even slightly promoted. Most likely, the OTC concentration of the R2 group had little effect on the activity of methanogens, and OTC was used as a carbon source for microorganisms in the anaerobic process [18].

A Gompertz modified equation was used to simulate the cumulative methane production curve. The key simulation parameters of the maximum methane production potential (M_m), maximum rate of methane production (R_m), lag period (λ), and correlation coefficient (R^2) are shown in Table 2. The values of R^2 were all >0.997 , indicating that the cumulative methane yields fit the Gompertz modified model. The potential maximum methane production G_{max} followed the order $R2 (1523.78 \pm 14.02 \text{ mL}) > R1 (1501.92 \pm 13.07 \text{ mL}) > R3 (1450.83 \pm 20.01 \text{ mL}) > R4 (1306.06 \pm 14.02 \text{ mL})$. The maximum rate of methane production R_m followed the order $R1 > R2 > R4 > R3$, indicating that an OTC concentration of 0.1 mg/L had no inhibitory effect on the methanogenesis and methanogenesis rate. In contrast, OTC levels of 0.5 and 1 mg/L had a certain in-

hibitory effect on methanogenesis and, consequently, the methanogenesis rate. In the experimental concentration range, higher OTC contents had higher inhibitory impacts on biogas production.

Table 2. Key kinetic parameters during anaerobic methane production for reactor groups R1–R4.

Key Parameters	R1 (0 mg/L)	R2 (0.1 mg/L)	R3 (0.5 mg/L)	R4 (1.0 mg/L)
Mm (mL)	1501.92 ± 13.07	1523.78 ± 14.02	1450.83 ± 20.01	1306.06 ± 14.02
Rm (mL/d)	122.99 ± 3.90	119.64 ± 3.99	103.29 ± 4.09	104.41 ± 4.01
R ²	0.998	0.998	0.997	0.997
λ (d)	3.51 ± 0.19	3.25 ± 0.21	4.20 ± 0.26	3.87 ± 0.23
Mm (mL)	1501.92 ± 13.07	1523.78 ± 14.02	1450.83 ± 20.01	1306.06 ± 14.02
Rm (mL/d)	122.99 ± 3.90	119.64 ± 3.99	103.29 ± 4.09	104.41 ± 4.01

Note: Mm = maximum cumulative methane volume (mL), Rm = maximum methane production rate (mL/d), and λ (d) = lag time.

Similar findings have been reported in the literature. For example, Yin indicated that the value of the Rm parameter decreased as the concentration of CTC or OTC in the sludge increased [17]. In another study [19], the authors showed that an OCT concentration of 3.1 mg/L could reduce the production of CH₄ by 27%. However, another study found no significant inhibitory effect at OTC concentrations of 30–90 mg/L [9]. These inconsistencies can be attributed to differences in the history of the sludge used and the operating conditions, including inoculum and manure sources, the inoculum/fertilizer ratio, antibiotic concentration, reactor size, and batch experiment or continuous operation [2]. In the current work, the effects of OTC at 0.5 and 1.0 mg/L were obvious, which implied that these concentrations would prolong the lag phase and have a certain inhibitory effect on methane production.

3.2. Effect of OTC on pH and VFA Production from Anaerobic Digestion

The pH variations along the digestion process are shown in Figure 3f. The initial pH values for all reactors ranged between 7.42 and 7.60, which are considered suitable for anaerobic digestion [20]. The pH value of each reactor gradually increased within the first 3 h, most likely because the anaerobic system entered the first stage of anaerobic fermentation, and nitrogen-containing compounds such as protein and urea began to be hydrolyzed and fermented by the fermenting bacteria to form ammonia nitrogen [21], leading to an increase in the pH. Subsequently, the pH of each reactor gradually decreased to about 6.5, probably because the anaerobic reaction system converted the intermediate products produced in the early stage into VFAs, H₂, and CO₂ under the action of hydrogen-producing acetogens [22]. The reaction system begins to produce and accumulate acids, causing the pH to drop slowly. In the following phase, the methanogens use, for example, acetic acid or methanol as a substrate and convert it into methane, thereby reducing the acidity in the system [22]. In addition, anaerobic microorganisms convert a large amount of organic nitrogen into ammonia nitrogen, which is promoted under high temperatures. The increase in alkalinity of the anaerobic digestion fluid can effectively neutralize the acid generated and increase the pH value of the digestion fluid. Therefore, the pH of each reactor began to rise gradually after the fifth day. The pH values of R1, R2, R3, and R4 increased from 6.70, 6.83, 6.81, and 6.82 to 8.36, 8.42, 8.37, and 8.48, respectively. Similarly, Yin found that after the methanogenesis stage, the final pH of the digestion solution increased from 7.7–7.9 to 8.2–8.3 [18]. In a similar study, high concentrations of antibiotics caused a sharp decrease in pH in the initial anaerobic stage [23]. However, the OTC concentrations used in our experiment were relatively low, and the pH change trends of all reactors were roughly the same. The pH of each group was between 8.3 and 8.5 after digestion, indicating that the reaction system was relatively stable.

Figure 3. Changes of volatile fatty acid (VFA) components in (a) R1 (control test), (b) R2 (with 0.1 mg/L oxytetracycline (OTC)), (c) R3 (with 0.5 mg/L OTC), and (d) R4 (with 1 mg/L OTC), as well as the changes in total VFAs (e) and pH value (f) of R1–R4.

VFAs are intermediate compounds formed during the anaerobic degradation of manure [18]. In this study, acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid were measured (Figure 3a–d). The total VFAs produced in the reactor are shown in Figure 3e. The VFA concentration in all reactors first increased and then decreased, reaching the maximum on day 1 at 1346.94, 1160.71, 1850.14, and 2232.79 mg/g

VS, respectively. The increase in VFA production and the early peaks may have been due to the dissolution and hydrolysis of organic matter [24], and high temperature conditions can accelerate this process. For example, in another study, the VFAs of the anaerobic system with roxithromycin reached the maximum on day 3 (control group) and day 6 (experimental group) under moderate temperatures and anaerobic conditions [25]. At the stage of acid production (1 d), the increase in VFA production was mainly due to the increase in acetic acid and butyric acid, which was similar to previous findings with sulfadiazine and chlortetracycline [18,26]. This leads us to infer that the type of anaerobic fermentation of swine manure and sludge was butyric acid-type fermentation [15], and the concentration of butyric acid accounted for more than 80% of the total concentration of VFAs. The maximum VFA concentrations of R1 and R2 were 1346.94 and 1160.71 mg/g VS, respectively, indicating that 0.1 mg/L OTC had little effect on the VFA changes in the anaerobic system. The maximum VFA concentrations in the R3 and R4 reactors were 1850.14 and 2232.79 mg/g VS, respectively, and 1.37 and 1.65 times than that of the control, respectively, indicating that 0.5 and 1 mg/L OTC can promote the production of VFAs. From day 2 to day 7 of digestion, the concentration of total VFAs in each reactor decreased, along with the levels of butyric acid, while the acetic acid concentration gradually increased. Most likely, in the acetylation stage of the system, VFAs were further converted to acetic acid, carbon dioxide, and hydrogen by acetogenic bacteria [27]. This process consumes the acid accumulated in the system and, at the same time, butyric acid is converted into acetic acid. Therefore, the concentration of butyric acid decreases while that of acetic acid gradually increases. From day 1 to day 5, the propionic acid concentrations of R3 and R4 were significantly greater than that of the control group. For example, the concentration of R1 propionic acid was 177.12 mg/g VS, while those in R3 and R4 were 294.25 and 327.91 mg/g VS on the first day, respectively. Similarly, sulfadiazine has been reported to increase the concentration of propionic acid from anaerobic fermentation [26]. One study stated that propionic acid is an unfavorable substrate for microorganisms, and getting the degradation reaction of propionic acid to proceed spontaneously is difficult. This is because propionate degradation is initially limited by thermodynamics, as the Gibbs free energy for the conversion of propionic acid to acetate is 76 kJ/mol, and therefore, this reaction cannot occur spontaneously [28]. The accumulation of propionic acid in VFAs is directly related to the processing capacity of the anaerobic digestion system. Such accumulation will lead to a decrease in the gas production of the system, which is usually a sign of system unbalance [29].

In our study, OTC at concentrations of 0.5 and 1.0 mg/L could promote the accumulation of propionic acid in the system, thereby promoting the transformation from butyric acid fermentation to propionic acid fermentation. This may, in turn, inhibit the degradation of propionic acid, causing it to temporarily accumulate in the reaction system, thereby inhibiting the anaerobic digestion system. When the digestion is complete, most of the VFAs are degraded, which is in agreement with previous findings [18]. In our experiment, the highest degradation rates were 0.76, 0.74, 0.92, and 0.85. However, propionic acid was still the main component of VFAs, most likely because propionic acid was difficult to degrade.

The VFA concentrations of each reactor after the reaction were 244.36, 298.61, 152.18, and 365.81 mg/g VS, without any significant differences among the final VFA contents of each reactor. In summary, compared with the control group, the other three reactors showed no significant impact on butyrate-type fermentation, although in R3 and R4, the accumulation of propionic acid was affected. Group R2 (0.1 mg/L OTC) showed no significant effect on VFAs, while R3 (0.5 mg/L OTC) showed an increased peak value of VFAs and a promoted temporary accumulation of propionic acid; however, VFAs were not accumulated. Group R4 (1.0 mg/L OTC) also showed an increased peak value of VFAs and promoted the temporary accumulation of propionic acid. At the end of the reaction, the VFA concentration was slightly higher than that of the control group with insignificant accumulation.

3.3. Effects of OTC on the Solubilization Process and the Entire Anaerobic Reaction

Solubilization refers to the incipient phase of anaerobic fermentation and is commonly considered as a speed-limiting step [30]. Once the solid releases more dissolved total organic carbon (TOC) into the solution, more bioavailable substrates will be produced. As shown in Figure 4a, the TOC concentrations of R1–R4 were 4850.8, 4759.3, 4925.6, and 4898.2 mg/L after 3 h of the reaction, respectively. On the second day, these values were 5000.2, 5193.4, 5490.5, and 5259.8 mg/L, following the order R3 > R4 > R2 > control. Lower TOC values in the control (R1) indicate that the addition of OTC can increase the concentration of TOC.

Figure 4. Changes in total organic carbon (TOC) (a) and OTC concentration (b) in the liquid phase of groups R1–R4.

The changes in DOM in the fermentation broth were further analyzed by EEM fluorescence spectroscopy, and the results are shown in Figure 5a–d. We observed one major fluorescence peak from the fluorescence spectra, located at Ex/Em 260–280/250 (signed as Peak A). The peak size of each reactor was 1465, 2010, 2580, and 2570, respectively. Previous studies have shown that Peak A is geared toward tyrosine substances, which are generally biodegradable [31]. Compared with the control, in all treatments, the fluorescence intensities of Peak A were significantly increased, following the order R3 > R4 > R2 > control. The continuous release of more organic substrates into the fermentation broth may lead to increased fluorescence intensity [32]. Our results show that OTC increased the biodegradability of soluble organic matter and facilitated its dissolution, thus providing more soluble organic matter as substrate for the production of VFAs.

Figure 5. Changes in components (after 3 h) in (a) R1 (control test), (b) R2 (with 0.1 mg/L OTC), (c) R3 (with 0.5 mg/L OTC), and (d) R4 (with 1 mg/L OTC).

These results were consistent with previous findings, which may show that the presence of antibiotics can expedite the release of extracellular polymeric substances (EPS), thus promoting cell rupture and increasing the production of dissolved organic matter [25,31]. EPS are high-molecular polymers excreted by microorganisms. As the main components of EPS, proteins provide a binding site for bacteria [33]. Functional groups (such as the carboxyl, amine, and hydroxyl groups) and hydrophobic regions on the proteins can bind to toxic substances (for example OTC), accompanied by the release of large amounts of EPS. As a consequence, the presence of OTC expedited the release of EPS. In addition, EPS can be bound with toxic substances, resulting in lower concentrations of toxic pollutants in the external environment [24,34]. In terms of the degradable substrate producing VFAs, solubilization is generally considered a rate-limiting step of anaerobic digestion. Once solubilization is enhanced, more substrate can be produced to produce VFAs, which is very important and beneficial for subsequent digestion processes. The rupture of these cells is key to the solubilization process and thus affects the production of VFAs [25,30].

Based on these results, the effect of 1.0 mg/L OTC on the entire process of the anaerobic reaction system may be as follows (Figure 6). First, it promotes the rupture of cells, thereby enhancing the biodegradability of soluble organic matter and increasing the production of soluble organic matter. Second, the formation of more soluble organic matter provides conditions for the formation of VFAs, which in turn promotes acidification. Finally, to a certain extent, the activity of microorganisms is inhibited, the production of methane is inhibited, the lag period of methane is prolonged, and VFAs temporarily accumulate in the system.

Figure 6. Schematic representation of the effect of OTC on the anaerobic fermentation of swine manure.

3.4. Degradation of OTC during Anaerobic Digestion

Figure 4b shows the changes in the OTC concentrations in the anaerobic reaction system. At the beginning of the experiment, the OTC concentration dropped rapidly, reaching degradation rates of 73.29%, 88.75%, 96.93%, and 97.05% at the end of the experiment for R2, R2, R3, and R4, respectively. A previous study on OTC removal during the anaerobic digestion of swine manure obtained similar values [35]. These results suggest that thermophilic anaerobic digestion has a high degradation ability for OTC, which increases with increasing OTC concentrations within the experimental range.

The degradation of OTC follows the first-order kinetic Equation (3), except in the control group, and higher OTC amounts result in a better curve fit. The estimated parameters are presented in Table 3. The lowest degradation rate constant was estimated to be 0.6455 at day 1 for R2, whereas the highest degradation rate constant was 1.3698 at day 1 for R4. Our resulting degradation rate was therefore higher than those found in previous studies. For example, the degradation rate of OTC (10 mg/L) under medium temperature conditions, studied by Alvarez, was 0.052 [2], most likely because high temperatures accelerated the degradation and adsorption of OTC. Similarly, Wu pointed out that the degradation rate of tetracycline in swine manure increased with increasing temperatures, reaching a maximum at 55 °C [36]. The degradation rate was generally higher in the reactor with a higher OTC concentration, indicating the effect of bioavailability on OTC degradation [2]. In this study, the significant OTC removal may be due to biodegradation and sludge adsorption, which are the main antibiotic removal methods in the biological process [18]. The OTC contains OH active groups that provide a graft site, making it biodegradable [17]. In addition, OTC can also be degraded during the acidogenic process because the dimethylamine in OTC can isomerize with a reversible reaction under acidic conditions [17].

Table 3. Fitting results of the degradation kinetics of swine manure in the anaerobic process using a simple first-order kinetic model.

Key Parameters	R1 (0 mg/L)	R2 (0.1 mg/L)	R3 (0.5 mg/L)	R4 (1.0 mg/L)
k (d ^{−1})	/	0.65	0.98	1.37
R ²	/	0.70	0.82	0.91
Degradation efficiency	0.73	0.89	0.97	0.97

4. Conclusions

Previous studies have found that OTC addition to anaerobic reactor systems (0.1, 0.5, and 1.0 mg/L) can promote cell rupture, thereby increasing the production of dissolved organic matter DOM and TOC. Group R2 (0.1 mg/L OTC) showed no significant effect on methane production and VFA accumulation, whereas R3 (0.5 mg/L OTC) and R4 (1.0 mg/L OTC) could inhibit methane production and prolong the lag period to a certain extent. The inhibition rate of methane accumulation was divided into 4.03% and 14.12%. At the same time, the peak value of VFAs increased by 37% and 65% with the temporary accumulation of propionic acid. Group R3 (0.5 mg/L OTC) showed no accumulation of VFAs, and R4 (1.0 mg/L OTC) had small but insignificant VFA accumulation in the final stage.

The overall effect of 0.1 mg/L OTC on the anaerobic reaction system may be the promotion of cell rupture, thereby promoting the production of dissolved organic matter.

Soluble organic matter provides conditions for the formation of VFAs, which in turn promotes acidification. Finally, the methanogen was suppressed to a certain extent by OTC, which reduced methane production and caused the temporary accumulation of VFAs.

The research results of this article can provide a theoretical basis and reference for the high-temperature anaerobic digestion of pig wastewater containing OTC.

Author Contributions: Conceptualization, Z.F. and M.Z.; methodology, Z.F. and X.L.; software, Z.F.; validation, Z.F. and Q.S.; formal analysis, Z.F.; investigation, Z.F. and X.C.; data curation, Z.F. and C.J.; writing—original draft preparation, Z.F.; writing—review and editing, Z.F. and X.L.; visualization, Z.F. and Z.H.; supervision, X.L.; project administration, X.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Major Science and Technology Program for Water Pollution Control and Treatment (Grant No.2017ZX07602-001).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank National Major Science and Technology Program for Water Pollution Control and Treatment (Grant No.2017ZX07602-001) and ABA Chemicals for their support and donations.

Conflicts of Interest: The authors declare no conflict of interest.

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