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Effects of oxytetracycline on mesophilic and thermophilic anaerobic digestion for biogas production from swine manure

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ABSTRACT

Anaerobic digestion (AD) technology is a valuable method for producing biogas fuels and treating livestock wastes such as swine manures concurrently. However, the effect of emerging antibiotics on the AD process is still undiscovered. In this study, the influence of oxytetracycline (OTC) on the AD process was investigated under mesophilic (35 ± 0.5 °C) and thermophilic (55 ± 0.5 °C) conditions, respectively. The presence of OTC significantly inhibited the production of methane in AD process, where the methane yields decreased by 58.6% and 73.3% in mesophilic and thermophilic ADs when the initial concentration of OTC was 400 mg/L, respectively. Besides, OTC can be markedly degraded by the AD process with a removal efficiency higher than 90% when the OTC initial concentration is lower than 10 mg/L. Furthermore, a higher concentration OTC led to a lower biomethane yield, energy conversion efficiency, and contaminant removal during both mesophilic and thermophilic ADs. With adding of 400 mg/L OTC, *Clostridium sensu stricto 1* (32.9%) and *Anaerolinea* (29.3%) are dominant to biodegrade organic matter during mesophilic and thermophilic AD systems. Correspondingly, *Methanosaeta* was functional in producing biomethane in both mesophilic (60.8%) and thermophilic (56.4%) AD systems. Additionally, *Methanolinea* was bearable to high concentrations of OTC during mesophilic and thermophilic AD processes.

1. Introduction

The livestock and poultry breeding industry that provides meat, eggs, and milk for people has become increasingly large-scale, intensive, and industrialized with the improvement of people's living standards and the growth of living needs recently [1]. China has produced much livestock and poultry manure with a continuously increasing breeding scale [2]. Pigs account for about 60% of the total livestock, and swine waste is the primary source of livestock and poultry manure [3]. Dealing with these feces without proper treatment will cause air, water, and soil pollution [4].

As a mature technology, anaerobic digestion (AD) has been widely used in treating livestock and poultry manure, meanwhile producing biofuels such as biomethane and biohydrogen [5–7]. These biofuels can be extensively used in daily life and process industries. Temperature is an essential factor in the AD process [8], where the changeable temperature will affect the metabolic activity of methanogens, thereby affecting methane production efficiency and reducing the performance of sewage pollutant removal. Methanogens can be divided into mesophilic and thermophilic methanogens with temperature ranges at mesophilic 30–40 °C and thermophilic 50–60 °C [9]. Methanogens are extremely sensitive to temperature, and small fluctuations in temperature may have an enormous impact on the digestion system [10]. Hence a slight increase in temperature could improve the activity of methanogens and enhance biogas production. When comparing with the medium temperature condition, the biogas production from hog waste under the thermophilic condition increased by about 55.6% because the thermophilic anaerobic methanogens had higher metabolic activity, thus leading to higher methane yield and organic matter degradation rate [11].

Antibiotics are widely used in the pig industry as growth promoters and disease-prevention substances [12]. The average consumptions of antibiotics per kilogram of cattle, chickens, and pigs are 45 mg/kg, 148 mg/kg, and 172 mg/kg, respectively [13]. Specifically, tetracycline is the most commonly used antibiotic in swine production worldwide [14].

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Nomenclature

AD	Anaerobic digestion
OTC	Oxytetracycline
TS	Total solids
VS	Volatile solids
IC	Inhibition coefficient
$APR_{90\%}$	Average gas production rate when the gas production reaches 90%
HHV	High heating value
ECE	Energy conversion efficiency
COD	Chemical oxygen demand
NH_4^+-N	Ammonia nitrogen
M-A	Sludge before mesophilic AD
M-B	Sludge after mesophilic AD without OTC
M-C	Sludge after mesophilic AD with 400 mg/L OTC
T-A	Sludge before thermophilic AD
T-B	Sludge after thermophilic AD without OTC
T-C	Sludge after thermophilic AD with 400 mg/L OTC

Oxytetracycline (OTC) is one of the most typical antibiotics among tetracycline, and its content in livestock and poultry manure could achieve as high as 600–1000 mg/L [15,16].

Treating antibiotics via AD technology has attracted increased attention recently. Kasumba et al. [17] found that 64–88% of chlortetracycline was removed via AD of poultry manure (containing 1 mg/L chlortetracycline). On the other hand, sulfadiazine was confirmed to inhibit fatty acid hydrolysis and methanogenesis during the cow manure AD process [18]. Zhao et al. [19] found that the degradation of sulfamethoxazole mainly relied on biodegradation, and the removal rate was as high as 86%. Most current research focuses on trace antibiotics, but there is little research on OTC, which accounts for a large proportion of antibiotics in livestock and poultry manure. A systematic study will support utilizing livestock wastes containing antibiotics via AD technology.

In this study, swine manure mesophilic and thermophilic ADs were conducted to explore their biofuel production and OTC removal abilities. The swine manure containing OTC in different concentrations was treated with AD, and the removal efficiency of OTC via the AD process was investigated. Subsequently, the degradation characteristics of OTC under mesophilic and thermophilic conditions were compared. Additionally, the influences of OTC on anaerobic bacterial and archaeal communities in digestion systems were analyzed.

2. Materials and methods

2.1. Materials

The granule sludge used in the experiment was collected from the secondary sedimentation tank of the municipal sewage treatment plant (Weifang city, Shandong province, China). The initial inoculum was activated and enriched with 2 g/L β-cellulose three cycles in an anaerobic environment at 35 °C, and the time interval for every cycle was 7 d. The thermophilic microbial communities were enriched with 2 g/L β-cellulose in an anaerobic environment with a gradient increasing temperature from 35 ± 0.5 °C to 55 ± 0.5 °C with 2 °C increases each time for 2 days and finally enriched at 55 ± 0.5 °C for 7 days.

Swine manure was taken from the countryside in Chongqing, China. The fresh swine manure was dried, crushed, and stored in a dry apparatus. The tested swine manure had 85.2% total solids (TS) and 59.3% volatile solids (VS). The C, O, H, N, and S elemental percentages of dry swine manure were about 33.2%, 59.1%, 4.3%, 2.8%, and 0.6%, respectively. The OTC (greater than 98% purity) was purchased from Macklin Biochemical Co., Ltd (Shanghai, China).

2.2. Mesophilic and thermophilic anaerobic digestion

The mesophilic AD experiments were carried out in 250 mL digestion flasks at 35 ± 0.5 °C. The inoculum of activated sludge was 50 mL, and the added swine manure was 10 g VS/L in every flask. These digestion flasks were sealed with butyl rubber stoppers and purged with nitrogen for 10 min to ensure anaerobic environments. The gas of every digestion flask was collected in a graduated gas collector filled with saturated NaCl acidic solution with methyl orange as the indicator. The experimental flask containing only activated sludge was the blank group, and the flask without OTC was the control group. The OTC with concentrations of 1, 5, 10, 25, 50, 150, and 400 mg/L were added to anaerobic flasks to run experimental groups, and all experiments were triplicated. The thermophilic AD experiments were conducted at 55 ± 0.5 °C, and other conditions were consistent with the mesophilic AD process.

2.3. Analytical procedures

The methane content was tested by a Trace 1300 gas chromatography (GC, ThermoFisher, USA) with a ShinCarbon ST column (2 m, OD 1/16, ID 1.0 mm, Mesh 100/120). The methane yield (mL/g VS) was calculated based on the ratio of cumulative methane volume to the initial total organic load. The corresponding values are normalized to a standard pressure of 1 atm and temperature of 0 °C.

The inhibitory influence of OTC on methane production by AD was quantitatively analyzed by the inhibition coefficient (IC) [20], as shown in Eq. (1).

$$IC\ (\%) = \left(1 - \frac{APR_{90\%}\ with\ OTC}{APR_{90\%}\ without\ OTC}\right) \times 100 \tag{1}$$

$APR_{90\%}$ represents the average gas production rate when the gas production reaches 90% of the total cumulative production.

The high heating value (HHV) of methane is 889 kJ/mol [21], equivalent to 39.7 kJ/L CH_4. The HHV of swine manure (14.7 kJ/g) was calculated according to the Mendeleev equation [22], as shown in Eq. (2).

$$HHV\ (kJ/g) = 0.33858 \times C + 1.254 \times H - 0.10868 \times (O - S) \tag{2}$$

C, H, O, and S express the corresponding elements in swine manure based on VS content.

The energy conversion efficiency (ECE) was calculated from the ratio of methane HHV to initial substrate HHV, as shown in Eq. (3). It should be noted that the process energy consumptions, such as heat and electricity, were not considered in the calculation of ECE.

$$ECE\ (\%) = \frac{Total\ energy\ of\ produced\ methane}{Total\ energyin\ the\ initial\ organic\ matter} \times 100\% \tag{3}$$

The chemical oxygen demand (COD) and ammonia nitrogen (NH_4^+-N) contents in digestion suspension were measured by the potassium dichromate method and salicylic acid photometric method via DR3900 spectrophotometer and DRB200 heating digestion unit (Hach, USA) [23]. The acetate content in digestion suspension was tested by the Trace 1300 GC (ThermoFisher, USA) with an Agilent DB-FFAP column (30 m × 0.25 mm × 0.25 µm). The digestion suspension was centrifuged, acidized by 3 mol/L hydrochloric acid aqueous, and filtered via 0.22 µm filter membrane before acetate content analysis.

The OTC concentration in the digestion system was measured by a High-performance liquid chromatography-mass spectrometry (HPLC-MS 8060) with column EC-C18 (2.1 × 50 mm, 1.9 µm). The column temperature was 30 °C, and the used mobile phase was 0.1% formic acid aqueous and acetonitrile [23].

2.4. Microbial community analysis

The sludge before mesophilic AD (M-A), sludge after mesophilic AD without OTC (M-B), sludge after mesophilic AD with 400 mg/L OTC (M-C), sludge before thermophilic AD (T-A), sludge after thermophilic AD without OTC (T-B), and sludge after thermophilic AD with 400 mg/L OTC (T-C) were collected to analyze the microbial communities via the high-throughput 16S rRNA sequencing by Illumina HiSeq 2500 (MoBio Laboratories, Carlsbad, USA). The centrifuged digestion suspension was adequately rinsed with phosphate-buffered saline. The V3-V4 region bacterial and the V4-V5 region archaeal 16S rRNA genes were detected by the primer sequences 338F, 806R, and primer sequences Univ519F, Arch915R [23,24].

3. Result and discussion

3.1. Effects of OTC on methane production from anaerobic digestion

3.1.1. Mesophilic anaerobic digestion

The methane yield of mesophilic AD when different concentrations of OTC were incorporated is shown in Fig. 1a. Only a tiny amount of methane was produced on day 1, indicating that bits of low molecular organics in swine manure were directly used for methane production. The control group began rapidly producing gas with the continuous hydrolysis of complex organics into easily degradable low molecular organic matter. And the total methane yield increased significantly until day 10, began to stabilize, and reached 166.1 mL/g VS on day 12. Generally, the methane yield in the control group was in the range of the AD methane yield (135–308 mL/g VS) calculated in the IEA report with swine manure as the substrate [25], verifying that the methane yield of the control group in this study was at a normal level.

In Fig. 1a, once OTC was added in, it significantly affected the methane production of the mesophilic AD system, as antibiotics would directly influence the metabolic process of microorganisms. The lag period of AD (the time when methane yield reached 10% total methane production) did not change significantly, but the methane yield visibly decreased when 1 mg/L OTC was added. The digestion stabilized on day 10 with 151.4 mL/g VS of total gas production, adding 1 mg/L OTC. The decrease in methane yield might be due to limiting hydrolysis, acidification, and methane production processes in the AD system by destroying the microbial cell wall with the addition of OTC [26]. For instance, Tian et al. [27] confirmed that OTC inhibited propionate hydrolysis acidification and methanogenesis during mesophilic AD. Tetracycline, another widely used antibiotic, was proved to show no significant effect on solubilization, hydrolysis, and homoacetogenesis processes, while severely inhibiting the acidogenesis, acetogenesis, and methanogenesis processes [28]. Furthermore, the methane yield gradually decreased with the enhancement of OTC, which was similar to a previous study focused on tetracycline antibiotic wastewater in AD [29]. The decline of methane yield was evident when the OTC concentration was enhanced to 10 mg/L, indicating the increasing inhibition of OTC on methane production. However, the mesophilic methane yield slightly decreased when changed OTC between 10 and 150 mg, which implied that the inhibition tended to a stable level except for enhanced OTC to extremely high content.

The influence of OTC on the methane production rate is shown in Fig. 1b. The control group without OTC had a noticeable methane production rate on day 1. Its rate peaked at 29.7 mL/g VS/d on day 4 and gradually decreased from day 5 until the end of gas production on day 12. When added OTC into the AD system, the methane production rate was significantly reduced and peaked at a lower value (16.7–25.3 mL/g VS/d) between days 4–6. Furthermore, with the increase in OTC, the

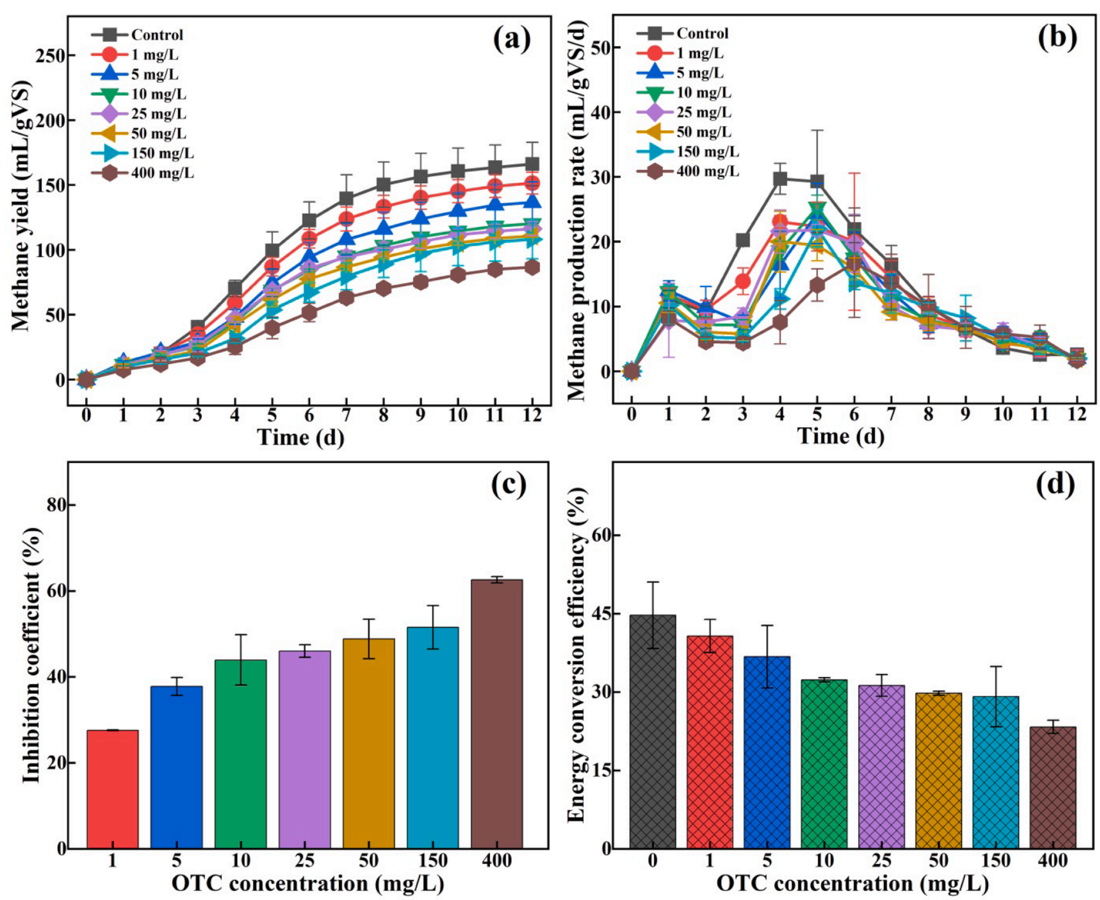

Fig. 1. Effects of OTC on mesophilic anaerobic digestion.

peak of methane production rate tended to move backward as more OTC resulted in higher stress to suppress anaerobic methanogenic communities [29]. Regarding the inhibitory coefficient of OTC on the AD system (Fig. 1c), the IC gradually increased from 27.6% to 62.6% with the enhancement of initial OTC concentration. The energy conversion efficiency of the AD system without OTC was 44.7%, comparable with the previously reported 49% of pig manure anaerobic digestion [30]. Meanwhile, the efficiency decreased to 23.3% with the improvement of initial OTC concentration (Fig. 1d), as the increased inhibition of OTC reduced methane production during the AD process.

3.1.2. Thermophilic anaerobic digestion

The influence of different concentrations OTC on the methane production of thermophilic AD is shown in Fig. 2a. The control group started to produce methane from day 1 and entered the methane production vigorous period on day 5. The methane production in the vigorous period was significantly higher than in mesophilic AD, which might cause by the high temperature accelerating the hydrolysis rate during anaerobic fermentation [31]. Ge et al. [32] found that the hydrolysis rate increased by 1.5 times for every 10 °C increase. The final methane production in the control group reached 245.6 mL/g VS with a higher ECE of up to 66.1% (Fig. 2d). However, thermophilic AD showed a relatively long lag period than mesophilic AD and gradually produced methane from day 3. It might relate to the high content of free ammonia in thermophilic AD (75–150 mg/L) than in mesophilic AD (lower than 10 mg/L), inhibiting enzymatic reactions of microbial cells and reducing growth rates of microorganisms [33–35]. Furthermore, the lag period was extended, and the methane yield was significantly reduced with the increase in OTC concentration (Fig. 2a), indicating the thermophilic microbial communities were more sensitive to OTC that their metabolisms were evidently impeded in the OTC environment initially.

The effect of OTC on the methane production rate of thermophilic AD is shown in Fig. 2b. The maximum methane production rate of the control group reached 42.6 mL/g VS/d, which was 1.4 times of the control group in the mesophilic system. It is mainly due to the strengthening of hydrolysis and acidification of macromolecular organics in the thermophilic process [31]. The peak of methane production rate shifted one day later but did not decrease when the initial OTC concentration was 1 mg/L, indicating that low concentrations of OTC showed slight inhibition on thermophilic AD. The maximum methane production rate tended to decrease and backward shift with increasing OTC concentration. Likewise, a previous study confirmed that the addition of 8 mg/L tetracyclines resulted in a reduction in the daily methane production to 19.5 mL/d [36]. Additionally, when added 0–10 mg/L OTC, the highest methane production rate in thermophilic AD was higher than that in mesophilic AD, since the thermophilic anaerobic flora with higher metabolic activities could reduce the inhibition of low concentration OTC [37,38].

The IC increased while ECE decreased gradually with increasing OTC in thermophilic AD (Fig. 2c and d). Significantly, the ECE of thermophilic AD with adding OTC of more than 150 mg/L was lower than that in mesophilic AD. It might relate to the significant decline of thermophilic anaerobic flora diversity at high concentrations OTC [39]. As well known that thermophilic AD always requires more heat than mesophilic AD to maintain the process. Therefore, mesophilic AD is assumed to be superior in energy conversion than thermophilic AD when OTC concentration is higher than 150 mg/L.

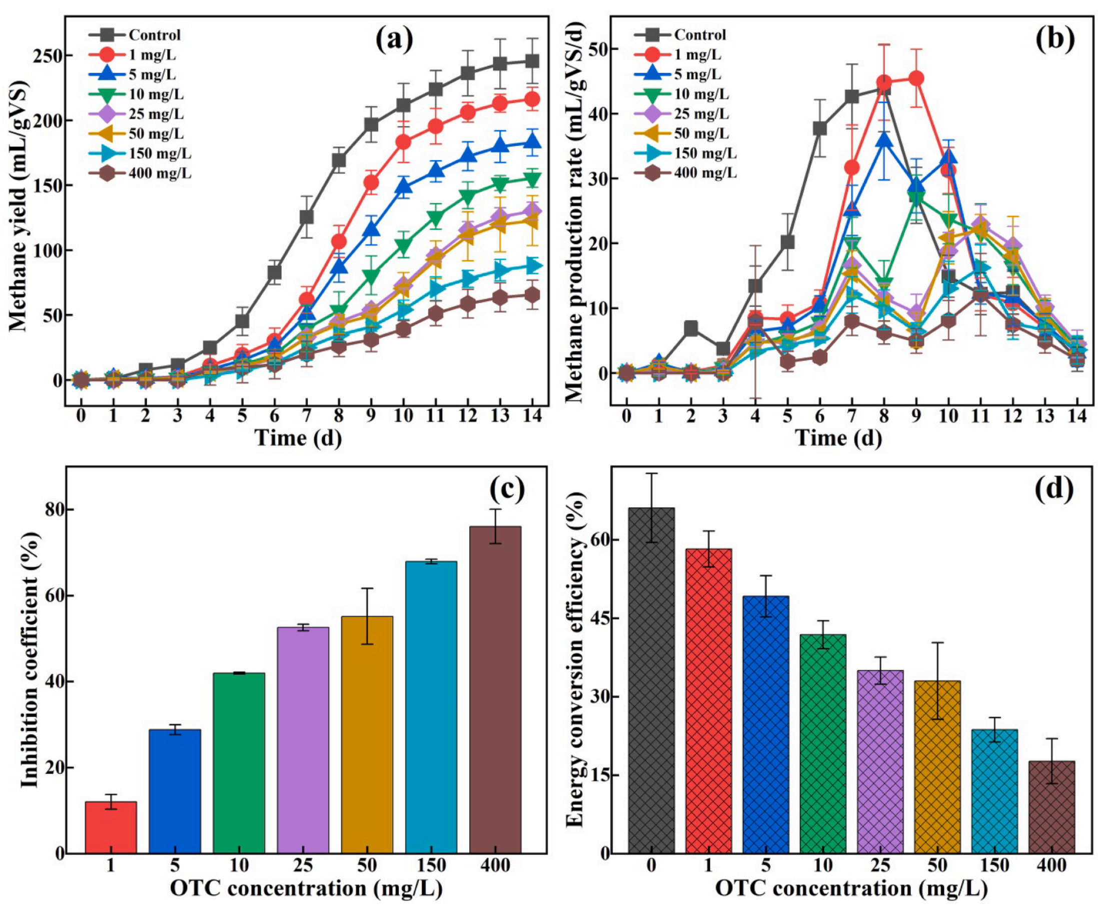

Fig. 2. Effects of OTC on thermophilic anaerobic digestion.

3.2. Effects of OTC on contaminant removal during anaerobic digestion

3.2.1. Mesophilic anaerobic digestion

The influence of different concentrations of OTC on removing COD by mesophilic AD is shown in Fig. 3a. The COD of the control group was significantly decreased with the prolonging of digestion, where the COD was reduced to 1520.4 mg/L with 80.0% COD removal efficiency. The consumption of organic matter in the AD system gradually fell off with the increase in OTC concentration because the activity of the flora in the system was inhibited by the addition of OTC [40]. For example, the final removal efficiency of COD was only 44% at a high concentration of OTC up to 400 mg/L. A previous study reported that the COD removal efficiency was decreased from 94% to 84% when adding 80 mg/L OTC in livestock anaerobic treatment [41]. Tetracycline, the same class as OTC, was also confirmed to significantly affect COD removal during the AD process [42].

As shown in Fig. 3b, the ammonia nitrogen in the control group gradually increased from 205.0 mg/L to 261.5 mg/L, with a 27.6% of increment. Swine manure contains many complex organics, where some ammonia nitrogen compounds were produced in the progress of hydrolysis and acidification of organics, increasing the ammonia nitrogen of the AD system [43,44]. The incorporation of a low concentration of OTC had almost no difference with the control group, while adding a high OTC caused a lower ammonia nitrogen value than the control group. The ammonia nitrogen only increased to 234.5 mg/L with adding 400 mg/L OTC, relating to the inhibition of hydrolysis and acidification processes of macromolecular organics such as proteins in AD by high concentrations of OTC [27,45].

The influence of OTC on the acetate content of mesophilic AD is shown in Fig. 3c. The higher initial concentration of OTC led to a lower acetate content in the early stage of AD, which might be due to the addition of OTC inhibiting the acetylation of small molecular organics, resulting in a decrease in acetate content [46]. Then the acetate content gradually increased as the AD progressed and began to decrease after reaching the peak of methane production rate with a slower declining rate at a higher initial OTC concentration. Significantly, some acetate remained after the end of AD, showing that the addition of OTC inhibited the conversion of acetate to methane [42].

3.2.2. Thermophilic anaerobic digestion

The influence of OTC on COD removal by thermophilic AD is shown in Fig. 4a. The COD with an initial value of 8450.9 mg/L was reduced to 750.1 mg/L, with the COD removal efficiency up to 91.1%. However, the removal of COD decreased significantly with the increase of OTC, as the thermophilic anaerobic flora were highly sensitive to OTC, and their activity was reduced with the addition of OTC [47]. The removal efficiency of COD was dropped to 44.6% when the initial OTC was as high as 400 mg/L, indicating that the activity of the flora in the AD system was seriously inhibited, which resulted in the reduction of organic matter consumption and the decline of methane production. This finding differed from a previous study that the COD removal efficiency kept around 40.9–44.4% when added 0–1000 mg/L OTC during the thermophilic AD [48], which might relate to the better bioactivity but weaker antibiotic-resistant ability of thermophilic flora used in this study.

The influence of OTC on the ammonia nitrogen content of thermophilic AD is shown in Fig. 4b. The ammonia nitrogen value of the control group increased from 205.0 mg/L to 319.5 mg/L, which increased by about 55.9%. The main reason was that the hydrolysis process of macromolecular organics in swine manure was strengthened under a

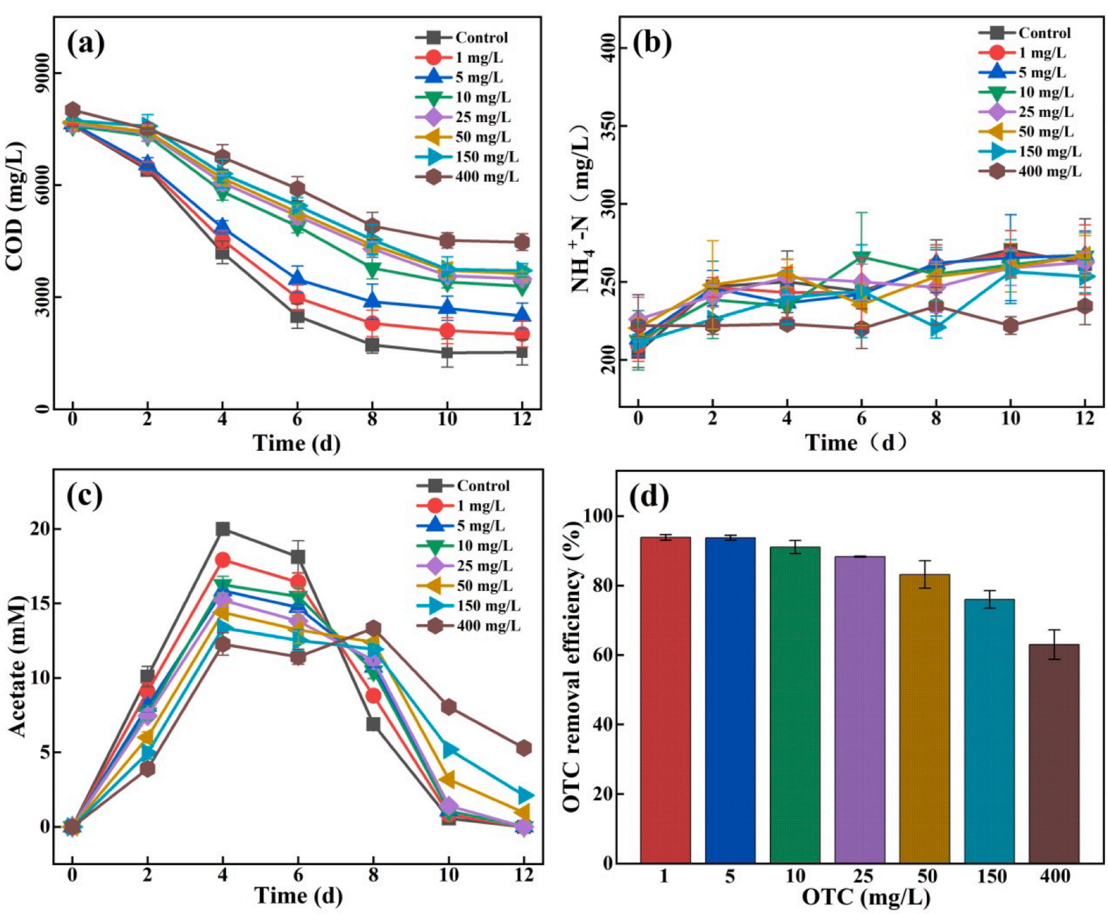

Fig. 3. Contaminant removal during mesophilic anaerobic digestion.

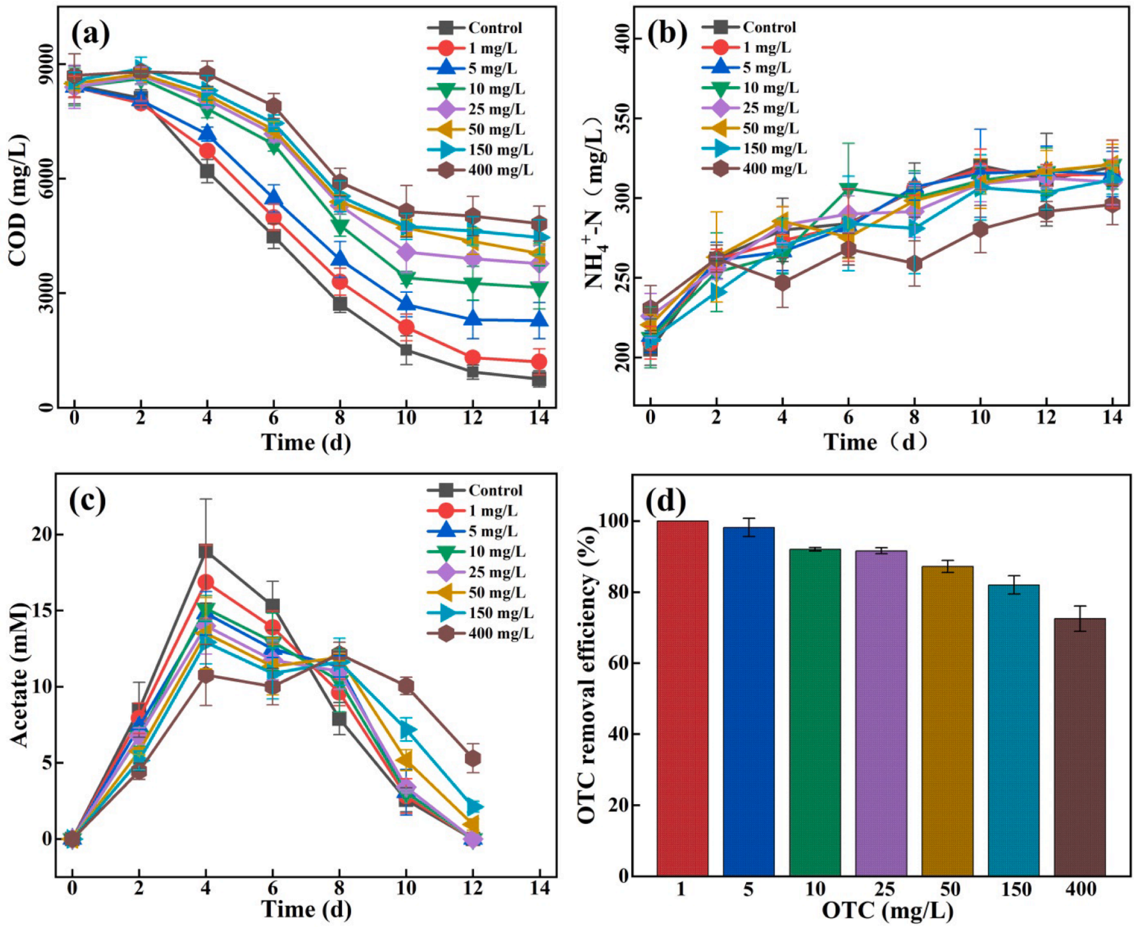

Fig. 4. Contaminant removal during thermophilic anaerobic digestion.

thermophilic environment, resulting in an increase in ammonia nitrogen [49]. In more detail, the change of ammonia nitrogen with adding 1–150 mg/L OTC in the thermophilic AD has no evident difference from that of the control group. The free ammonia molecules can diffuse through the microbial cell membrane [35], causing problems of changes in intracellular pH, increased energy requirements for maintenance, and inhibition of specific enzymatic reactions [34]. Therefore, the high content of free ammonia at a high temperature led to a long lag period in the early stage of the thermophilic process. The flora began to gradually produce methane after they adapted to the environment of high ammonia nitrogen and antibiotics in the AD system. However, the 400 mg/L OTC brought lower ammonia nitrogen content in the AD process, which might cause by the significant inhibition of thermophilic flora at high concentrations of OTC during the AD process.

The acetate content in thermophilic AD with adding different concentrations of OTC (Fig. 4c) increased at the beginning of four days and began to decline with the prolonging of AD. Same with mesophilic AD, the acetate content decreased in the initial stage at the higher initial concentration of OTC. Acetate was confirmed to remain in all working conditions after the finish of gas production. The higher amounts of OTC, the more residual acetates were detected, indicating that the methanogens activity was inhibited and resulted in the acetate accumulation under the inhibition of OTC.

3.3. Removal of OTC during the anaerobic digestion

The removal of antibiotics in mesophilic AD with the addition of OTC is shown in Fig. 3d. The OTC removal via AD was above 90% when the OTC initial concentration was less than 10 mg/L, where it reached 93.8% at 1 mg/L of OTC, and gradually decreased with the increase of OTC initial concentration. The removal of OTC in this study was higher than 59% of OTC removal (9.8 mg/L initial concentration) in a previous manure anaerobic digestion study [50], confirming the excellent capacity of the sludge to treat OTC. The removal rate declined to 63.0% with adding 400 mg/L OTC. OTC could be effectively removed by AD mainly via pathways of biosorption and biodegradation [51]. The extracellular polymeric substances of microbial cells containing abundant functional groups are significant in adsorbing OTC via interactions such as cation exchange and surface complexation [19]. On the other hand, co-metabolism is considered an essential pathway for the biodegradation of antibiotics in AD, where antibiotics were used as substrates and degraded by the flora metabolism [52]. It is assumed that the OTC would be adsorbed by anaerobic sludge first and ultimately biodegraded by microbial organisms second. The degradation of OTC during AD relies on the synergistic cooperation of various microbial groups, mainly associated with specific metabolic stages throughout the biodegradation process [53]. Thereby, the different initial concentrations of OTC were removed with different degrees.

The OTC was less than the 1 ng/L detection limit of HPLC-MS after the thermophilic AD when the initial OTC concentration was 1 mg/L, indicating the OTC was thoroughly removed. The removal of OTC was above 90% when the initial OTC concentration was lower than 10 mg/L, which gradually decreased with the increase of OTC concentration and was about 75.5% with an addition of 400 mg/L (Fig. 4d). The removal efficiency of OTC in thermophilic AD was higher than in mesophilic AD, mainly because the thermophilic anaerobic flora had more active metabolic activities [54], and the temperature increases resulted in a shorter degradation half-life of OTC [55]. For example, the degradation half-life of OTC in swine manure AD was dropped to 19.8 h at 40 °C from 84.7 h at 25 °C in a previous study [56]. Additionally, the hydrolysis of OTC was also significantly affected by temperature. Xuan et al. [57] found that the contribution of OTC hydrolysis at high temperatures in moist animal manure may become comparable and even more significant than that of biodegradation.

3.4. Microbial community analysis during anaerobic digestion

3.4.1. Mesophilic anaerobic digestion

Alpha diversity indexes for evaluating mesophilic flora richness and evenness are shown in Table 1. The Chao 1 indexes of bacteria and archaea in the M-B increased when compared with M-A, indicating the enrichment of microbial communities after AD treatment [24]. The Chao 1 index of the M-C with the addition of OTC was lower than those of the M-B. It demonstrated that OTC showed an inhibitory effect on microbial communities, resulting in a decrease in the metabolism of microbes. Therefore, the abundance of mesophilic anaerobic flora decreased. The Shannon and Simpson indexes of M-C were also lower than those of the M-B, implying that the diversity of mesophilic anaerobic flora decreased [58]. It showed that OTC could hinder the metabolism of microorganisms because some microorganisms that could not adapt to the OTC environment were eliminated.

The relative abundance of *Clostridium sensu stricto 1* significantly increased after the mesophilic AD (Fig. 5a), and it was the crucial bacteria for the biodegradation of organics in swine manure [59]. Additionally, the relative abundance of *Terrisporobacter* was also found to increase, which bacteria could convert carbohydrates into acetic acid and displayed a relationship with biogas production during AD [60]. *Syntrophobacter*, which could degrade acetate and propionate [61], declined in relative abundance from 5.8% to 1.1% after the AD process. *Candidatus Caldatribacterium*, another bacteria reported to function in producing acid and hydrogen [60], was also decreased in relative abundance. Significantly, the more OTC led to higher *Clostridium sensu stricto 1* and *Terrisporobacter* relative abundances, indicating that *Clostridium sensu stricto 1* and *Terrisporobacter* showed dominant properties to progress the biodegradation process than *Syntrophobacter* and *Candidatus Caldatribacterium* during the mesophilic AD system.

The archaea community analysis of mesophilic AD is shown in Fig. 5b, where *Methanogens,* such as *Methanosaeta*, *Methanobacterium*, *Methanomethylovorans*, *Methanolinea,* and *Methanomassiliicoccus,* were the dominant archaea. The relative abundance of *Methanosaeta* in the control group increased from 59.4% to 85.0% after mesophilic AD. It is an obligate anaerobic archaea, which is gram-negative and can use acetate to produce methane [62]. The relative abundance of *Methanosaeta* in the experimental group with adding OTC was lower than that in the control group, indicating that OTC inhibited its metabolism and resulted in limited acetate utilization by *Methanosaeta*. The relative abundances of *Methanomethylovorans* and *Methanolinea* in M-C were 10.0% and 10.2%, higher than those in M-B. *Methanomethylovorans* is a methylotrophic methanogen with an optimal temperature range of 25–50 °C. *Methanomethylovorans* can use methanol, monomethyl amine, dimethylamine, trimethylamine, dimethyl sulfide, and methyl mercaptan for catabolism [63]. The increase in relative abundance of *Methanomethylovorans* may be due to the formation of methylamine and other substances during the biodegradation of OTC. OTC is degraded via demethylation, deamidation, ring-cleavage, decarboxylation, and dehydroxylation reactions in biodegradation [16]. The methyl and amine compounds generated in this process can provide raw materials for the growth of *Methanomethylovorans*. *Methanolinea* is a hydrogen-consuming methanogen that can produce methane by using formate, H_2, or CO_2, which requires acetate or yeast extract for growing [64]. The increasing of *Methanolinea* relative abundance and decreasing of acetate content in the AD system indicated that the generated acetate tended to be consumed by *Methanolinea* when adding OTC in swine manure during the AD process.

3.4.2. Thermophilic anaerobic digestion

The species richness and evenness of thermophilic flora are shown in Table 2. The Chao 1 index in T-B increased compared with T-A after AD, indicating the enrichment of both bacteria and archaea [65]. The Chao 1 index in T-C tended to decrease with the addition of OTC, even lower than in T-A before AD. It demonstrated that OTC strongly inhibited the metabolism of thermophilic flora. Furthermore, the Shannon and Simpson indexes in T-C were also lower than those in T-A and T-B, which might be related to eliminating some flora that could not withstand the OTC under a thermophilic environment [58]. Compared with mesophilic flora (Table 1), the OTC inhibition of thermophilic flora was more severe than that of mesophilic flora.

The microbial community analysis of thermophilic AD is shown in Fig. 6. *Candidatus Caldatribacterium* (17.5%) and *Anaerolinea* (10.8%) were the dominant bacteria in T-A before thermophilic AD (Fig. 6a). The relative abundance of *Candidatus Caldatribacterium* in T-B and T-C decreased significantly in thermophilic AD, similar to that in mesophilic AD. *Anaerolinea* showed a lower relative abundance (6.2%) in T-B but a hugely higher relative abundance (29.3%) in T-C with the addition of 400 mg/L OTC after AD. *Anaerolinea* uses carbohydrates and proteins as substrates and can be co-cultured with methanogens [66]. The increase of *Anaerolinea* in T-C verified its strong adaptability toward OTC environments.

Regarding archaea communities (Fig. 6b), *Methanogens*, such as *Methanosaeta*, *Methanobacterium*, *Methanolinea*, *Methanomassiliicoccus*, *Methanosarcina*, and *Methanothermobacter*, dominated the archaea in thermophilic AD. The relative abundance of *Methanosaeta* increased from 44.6% to 61.2% in T-B and 56.4% in T-C after thermophilic AD, suggesting that high concentrations of OTC inhibited *Methanosaeta* during the thermophilic AD. The species level of *Methanolinea* was *Methanolinea* tarda NOBI-1, a thermophilic archaea. *Methanolinea* could utilize a wide range of substrates for metabolism, including acetate and methylated compounds [67]. The relative abundance of *Methanolinea* with the addition of OTC increased after thermophilic AD, while the *Methanolinea* in the control group almost disappeared. It might be caused by the methylated compounds formed from the biodegradation of OTC, which accelerated the enrichment of *Methanolinea* when OTC was incorporated into the AD system. Compared with mesophilic AD, the *Methanomethylovorans* had not been detected in thermophilic AD, while *Methanosarcina* and *Methanothermobacter,* which had not appeared in mesophilic AD, were discovered in thermophilic AD. It demonstrated

Table 1
Richness and evenness of mesophilic microbial community.

	Sample	Chao 1	Observed species	Shannon	Simpson	Coverage
Bacteria	M-A	1116.43	1017	6.05	0.95	1
	M-B	1430.88	1322	6.77	0.97	1
	M-C	1346.00	1190	6.31	0.96	1
Archaea	M-A	67.88	68	2.12	0.62	1
	M-B	72.20	66	2.12	0.61	1
	M-C	69.75	66	1.07	0.28	1

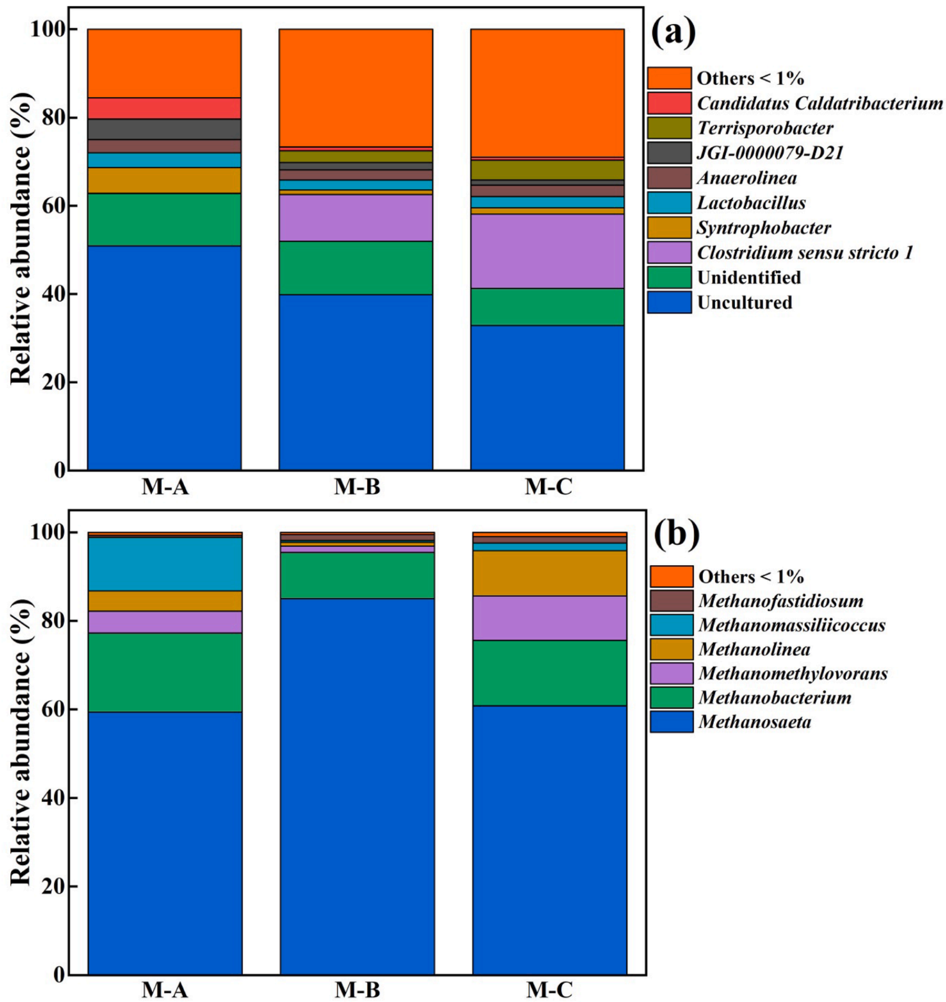

Fig. 5. Microbial community analysis of sludge before mesophilic anaerobic digestion (M-A), sludge after mesophilic anaerobic digestion without OTC (M-B), and sludge after mesophilic anaerobic digestion with 400 mg/L OTC (M-C) at genus level.

Table 2
Richness and evenness of thermophilic microbial community.

	Sample	Chao 1	Observed species	Shannon	Simpson	Coverage
Bacteria	T-A	738.27	660	6.25	0.96	1
	T-B	777.49	714	6.87	0.98	1
	T-C	613.77	570	5.43	0.91	1
Archaea	T-A	82.17	82	2.61	0.74	1
	T-B	106.25	80	2.27	0.64	1
	T-C	70.23	66	1.86	0.58	1

that *Methanosarcina* and *Methanothermobacter* were more bearable to high-temperature environments [68], while *Methanomethylovorans* was tolerable in mesophilic OTC environments but unconformable to thermophilic conditions [69].

3.5. Perspectives of manure anaerobic digestion with antibiotics

The presence of OTC (1–400 mg/L) in swine manure inhibited the AD process. Avoiding the inhibition caused by OTC to improve the recycling efficiency of manure waste is fundamental in AD technology. Firstly, it would be helpful to reduce the use of antibiotics in livestock and poultry to decrease the antibiotic content in manure. Secondly, systematic studies about AD processes containing different antibiotics should be conducted to confirm their synergistic influence on AD processes. Next, the swine manure AD process with OTC less than 1 mg/L should be further studied, as several swine manure contained OTC lower than 1 mg/L. Then, different AD schedules can be selected according to the antibiotics concentrations to improve biofuel yield and pollutant treatment efficiency. For example, thermophilic AD is more suitable when the OTC concentration in swine manure is less than 10 mg/L. The appropriate AD process should be selected after considering the equilibrium of energy consumption, biofuel yield, and OTC removal, when the OTC concentration is 10–150 mg/L. In contrast, the mesophilic process shows advantages when OTC concentrations are higher than 150 mg/L. Lastly, selecting microbial communities with strong adaptability and treatment capacity of antibiotics will be significant for developing the AD technology to reuse the livestock and poultry manure for biofuel production.

4. Conclusion

The addition of OTC with concentrations from 1 to 400 mg/L inhibited the AD process, where a higher OTC concentration would cause less biomethane yield, lower energy conversion efficiency, and reduced contaminants removal efficiencies. With adding 400 mg/L OTC, the ECE of mesophilic and thermophilic ADs were reduced to 23.3% and 17.7% from 44.8% and 66.1%, respectively. The OTC can be efficiently removed by mesophilic and thermophilic ADs, where 72.5–100.0% of OTC was removed via thermophilic AD, higher than 63.0–93.8% via

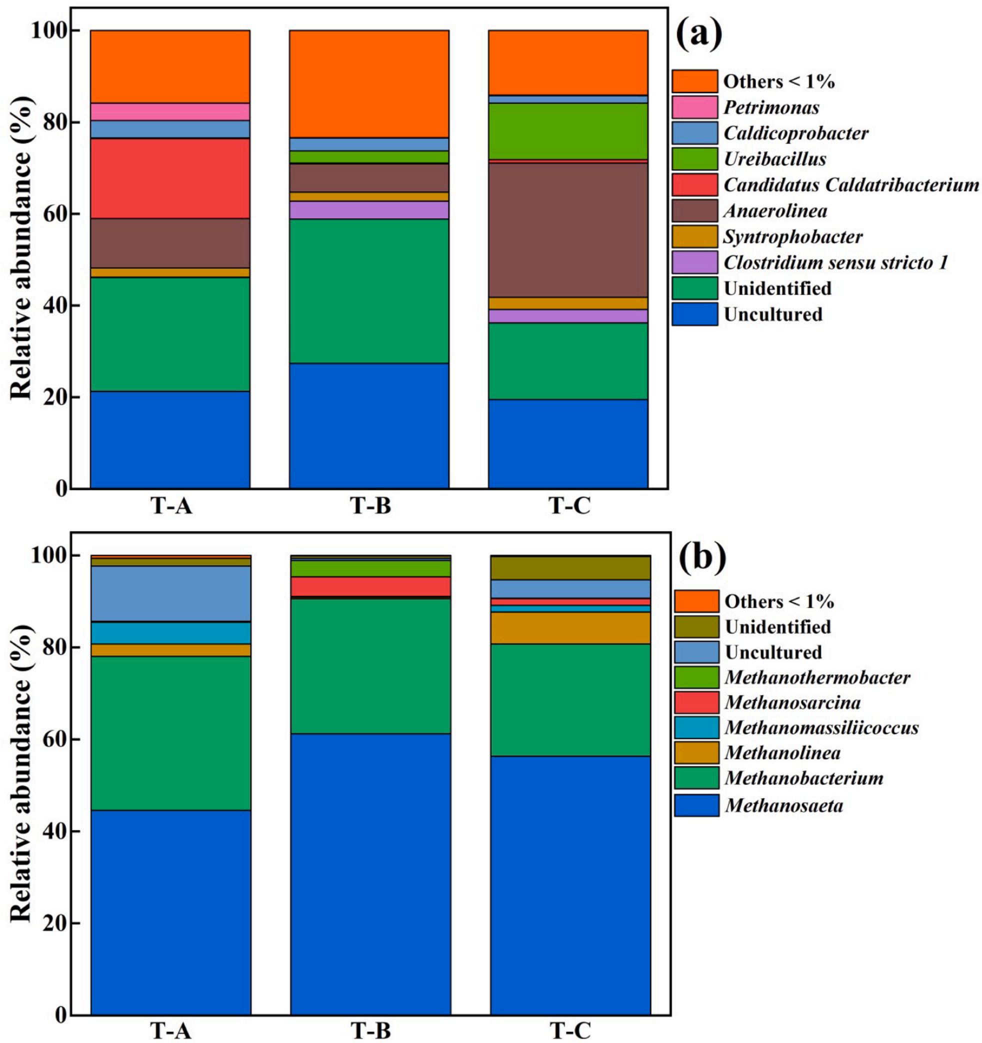

Fig. 6. Microbial community analysis of sludge before thermophilic anaerobic digestion (T-A), sludge after thermophilic anaerobic digestion without OTC (T-B), and sludge after thermophilic anaerobic digestion with 400 mg/L OTC (T-C) at genus level.

mesophilic AD. Additionally, *Clostridium sensu stricto 1*, *Methanomethylovorans*, and *Methanolinea* were bearable to OTC in high concentration during the mesophilic AD process, while *Anaerolinea* and *Methanolinea* were tolerable in an OTC environment during the thermophilic AD process.

CRediT authorship contribution statement

Jingmiao Zhang: Investigation, Data curation, Writing – original draft. **Shuai Wu:** Investigation, Data curation, Writing – original draft. **Ao Xia:** Conceptualization, Supervision, Writing – review & editing. **Dong Feng:** Investigation, Data curation. **Yun Huang:** Investigation. **Xianqing Zhu:** Investigation. **Xun Zhu:** Supervision. **Qiang Liao:** Writing – review & editing.

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.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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References

[1] Shim J-W, Pyo S, Lam SS, Jae J, Jeon B-H, Khan MA, et al. Catalytic pyrolysis of chicken manure over various catalysts. Fuel 2022;322:124241.
[2] Liu R, Xu F, Liu Y, Wang J, Yu W. Spatio-temporal characteristics of livestock and their effects on pollution in China based on geographic information system. Environ Sci Pollut Res 2016;23(14):14183–95.
[3] Wang Y, Zhang Y, Li J, Lin J-G, Zhang N, Cao W. Biogas energy generated from livestock manure in China: Current situation and future trends. J Environ Manage 2021;297:113324.
[4] Liu W-R, Zeng D, She L, Su W-X, He D-C, Wu G-Y, et al. Comparisons of pollution characteristics, emission situations, and mass loads for heavy metals in the

manures of different livestock and poultry in China. Sci Total Environ 2020;734:139023.
[5] Tao N, Xu M, Wu X, Pi Z, Yu C, Fang Di, et al. Supplementation of Schwertmannite improves methane production and heavy metal stabilization during anaerobic swine manure treatment. Fuel 2021;299:120883.
[6] Liew CS, Yunus NM, Chidi BS, Lam MK, Goh PS, Mohamad M, et al. A review on recent disposal of hazardous sewage sludge via anaerobic digestion and novel composting. J Hazard Mater 2022;423:126995.
[7] Deng C, Lin R, Kang X, Wu B, Wall D, Murphy JD. Improvement in biohydrogen and volatile fatty acid production from seaweed through addition of conductive carbon materials depends on the properties of the conductive materials. Energy 2022;239:122188.
[8] Ameen F, Ranjitha J, Ahsan N, Shankar V. Co-digestion of microbial biomass with animal manure in three-stage anaerobic digestion. Fuel 2021;306:121746.
[9] Lin Q, De Vrieze J, Li J, Li X. Temperature affects microbial abundance, activity and interactions in anaerobic digestion. Bioresour Technol 2016;209:228–36.
[10] Peces M, Astals S, Mata-Alvarez J. Response of a sewage sludge mesophilic anaerobic digester to short and long-term thermophilic temperature fluctuations. Chem Eng J 2013;233:109–16.
[11] Shaw GT-W, Liu A-C, Weng C-Y, Chou C-Y, Wang D. Inferring microbial interactions in thermophilic and mesophilic anaerobic digestion of hog waste. PloS One 2017;12(7):e0181395.
[12] Zhu X, Yuan W, Lang M, Zhen G, Zhang X, Lu X. Novel methods of sewage sludge utilization for photocatalytic degradation of tetracycline-containing wastewater. Fuel 2019;252:148–56.
[13] Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, et al. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci 2015;112(18):5649–54.
[14] Xu M, Li H, Li S, Li C, Li J, Ma Y. The presence of tetracyclines and sulfonamides in swine feeds and feces: Dependence on the antibiotic type and swine growth stages. Environ Sci Pollut Res 2020;27(34):43093–102.
[15] He Y, Tian Z, Yi Q, Zhang Yu, Yang M. Impact of oxytetracycline on anaerobic wastewater treatment and mitigation using enhanced hydrolysis pretreatment. Water Res 2020;187:116408.
[16] Wu S, Zhang J, Xia Ao, Huang Y, Zhu X, Zhu X, et al. Microalgae cultivation for antibiotic oxytetracycline wastewater treatment. Environ Res 2022;214:113850.
[17] Kasumba J, Appala K, Agga GE, Loughrin JH, Conte ED. Anaerobic digestion of livestock and poultry manures spiked with tetracycline antibiotics. J Environ Sci Health B 2020;55(2):135–47.
[18] Nurk L, Knörzer S, Jacobi HF, Spielmeyer A. Elimination of sulfonamides and tetracyclines during anaerobic fermentation-A "Cheshire Cat" phenomenon. Sustain Chem Pharm 2019;13:100157.
[19] Zhao Qi, Guo W, Luo H, Xing C, Wang H, Liu B, et al. Insights into removal of sulfonamides in anaerobic activated sludge system: Mechanisms, degradation pathways and stress responses. J Hazard Mater 2022;423:127248.
[20] Sun C, Liao Q, Xia Ao, Fu Q, Huang Y, Zhu X, et al. Degradation and transformation of furfural derivatives from hydrothermal pre-treated algae and lignocellulosic biomass during hydrogen fermentation. Renew Sustain Energy Rev 2020;131:109983.
[21] Xia Ao, Cheng J, Ding L, Lin R, Song W, Zhou J, et al. Enhancement of energy production efficiency from mixed biomass of *Chlorella pyrenoidosa* and cassava starch through combined hydrogen fermentation and methanogenesis. Appl Energy 2014;120:23–30.
[22] Madadi M, Bakr MA, Song G, Sun C, Sun F, Hao Z, et al. Co-production of levulinic acid and lignin adsorbent from aspen wood with combination of liquid hot water and green-liquor pretreatments. J Clean Prod 2022;366:132817.
[23] Zhang J, Xia Ao, Yao D, Guo X, Lam SS, Huang Y, et al. Removal of oxytetracycline and ofloxacin in wastewater by microalgae-bacteria symbiosis for bioenergy production. Bioresour Technol 2022;363:127891.
[24] Feng D, Xia Ao, Huang Y, Zhu X, Zhu X, Liao Q. Effects of carbon cloth on anaerobic digestion of high concentration organic wastewater under various mixing conditions. J Hazard Mater 2022;423:127100.
[25] Weinrich S, Schäfer F, Bochmann G, Liebetrau J. Value of batch tests for biogas potential analysis-Method comparison and challenges of substrate and efficiency evaluation of biogas plant. IEA Bioenergy; IEA Bioenergy. 2018. p. 10.
[26] Li Y, Chen Y, Wu J. Enhancement of methane production in anaerobic digestion process: A review. Appl Energy 2019;240:120–37.
[27] Tian Z, Zhang Y, Yang M. Chronic impacts of oxytetracycline on mesophilic anaerobic digestion of excess sludge: Inhibition of hydrolytic acidification and enrichment of antibiotic resistome. Environ Pollut 2018;238:1017–26.
[28] He D, Xiao J, Wang D, Liu X, Li Y, Fu Q, et al. Understanding and regulating the impact of tetracycline to the anaerobic fermentation of waste activated sludge. J Clean Prod 2021;313:127929.
[29] Zhang J, Chen G, Zhang Q, Luo Y, Wu C, Qin S. Adaption strategy of up-flow anaerobic sludge blanket reactor on tetracycline stress during tetracycline antibiotic wastewater treatment. Environ Eng Res 2021;27(3):200590–7.
[30] Clausen LR, Butera G, Jensen SH. Integration of anaerobic digestion with thermal gasification and pressurized solid oxide electrolysis cells for high efficiency bio-SNG production. Energy 2019;188:116018.
[31] Lin M, Ren L, Wandera SM, Liu Y, Dong R, Qiao W. Enhancing pathogen inactivation in pig manure by introducing thermophilic and hyperthermophilic hygienization in a two-stage anaerobic digestion process. Waste Manag 2022;144:123–31.
[32] Ge H, Jensen PD, Batstone DJ. Relative kinetics of anaerobic digestion under thermophilic and mesophilic conditions. Water Sci Technol 2011;64(4):848–53.
[33] Xia A, Cheng J, Murphy JD. Innovation in biological production and upgrading of methane and hydrogen for use as gaseous transport biofuel. Biotechnol Adv 2016;34(5):451–72.
[34] Jiang Y, McAdam E, Zhang Y, Heaven S, Banks C, Longhurst P. Ammonia inhibition and toxicity in anaerobic digestion: A critical review. J Water Process Eng 2019;32:100899.
[35] Rajagopal R, Massé DI, Singh G. A critical review on inhibition of anaerobic digestion process by excess ammonia. Bioresour Technol 2013;143:632–41.
[36] Liu W, Ning C, Sang R, Hou Q, Ni Y. Lignin-derived graphene quantum dots from phosphous acid-assisted hydrothermal pretreatment and their application in photocatalysis. Ind Crop Prod 2021;171:113963.
[37] Zhang K, Gu J, Wang X, Zhang X, Hu T, Zhao W. Analysis for microbial denitrification and antibiotic resistance during anaerobic digestion of cattle manure containing antibiotic. Bioresour Technol 2019;291:121803.
[38] Hu Z, Fan Z, Song Q, Khatoon R, Zhang M, Wang N, et al. The effect of exogenous oxytetracycline on high-temperature anaerobic digestion of elements in swine wastewater. Water 2021;13(24):3497.
[39] Lee J, Han G, Shin SG, Koo T, Cho K, Kim W, et al. Seasonal monitoring of bacteria and archaea in a full-scale thermophilic anaerobic digester treating food waste-recycling wastewater: Correlations between microbial community characteristics and process variables. Chem Eng J 2016;300:291–9.
[40] Chen X, Yang Y, Ke Y, Chen C, Xie S. Xie S. Sci Total Environ 2022;814:152852.
[41] Yan Z, Chen J, Liu Y, Shao J, Shu P, Wen S. Effects oxytetracycline on bacterial diversity in livestock wastewater. Environ Eng Sci 2017;34(4):265–71.
[42] Long S, Yang Y, Pavlostathis SG, Zhao L. Effect of sulfamethoxazole and oxytetracycline on enhanced biological phosphorus removal and bacterial community structure. Bioresour Technol 2021;319:124067.
[43] Jia Z, Ye X, Liu Y, Wang C, Cao C, Du J, et al. Metal-organic framework-derived porous metal oxide/graphene nanocomposites as effective adsorbents for mitigating ammonia nitrogen inhibition in high concentration anaerobic digestion of rural organic waste. Fuel 2023;332:126032.
[44] Qiang H, Wang F, Ding J, Zhang C. Co-digestion of swine manure and corn stalks with biochar as an effective promoter: An optimization study using response surface methodology. Fuel 2020;268:117395.
[45] Xiao L, Wang Y, Lichtfouse E, Li Z, Kumar PS, Liu J, et al. Effect of antibiotics on the microbial efficiency of anaerobic digestion of wastewater: A review. Front Microbiol 2021;11:611613.
[46] Fan Z, Zhang M, Chen X, Hu Z, Shu Q, Jing C, et al. 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(7):4014.
[47] Buhr H, Andrews J. The thermophilic anaerobic digestion process. Water Res 1977;11(2):129–43.
[48] Tian Z, Chi Y, Yu B, Yang M, Zhang Y. Thermophilic anaerobic digestion reduces ARGs in excess sludge even under high oxytetracycline concentrations. Chemosphere 2019;222:305–13.
[49] Yuan H, Zhu N. Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. Renew Sustain Energy Rev 2016;58:429–38.
[50] Arikan OA, Sikora LJ, Mulbry W, Khan SU, Rice C, Foster GD. The fate and effect of oxytetracycline during the anaerobic digestion of manure from therapeutically treated calves. Process Biochem 2006;41(7):1637–43.
[51] Zhou Q, Li X, Wu S, Zhong Y, Yang C. Enhanced strategies for antibiotic removal from swine wastewater in anaerobic digestion. Trends Biotechnol 2021;39(1):8–11.
[52] Zhou H, Cao Z, Zhang M, Ying Z, Ma L. Zero-valent iron enhanced in-situ advanced anaerobic digestion for the removal of antibiotics and antibiotic resistance genes in sewage sludge. Sci Total Environ 2021;754:142077.
[53] Cheng D, Ngo HH, Guo W, Chang SW, Nguyen DD, Liu Y, et al. Removal process of antibiotics during anaerobic treatment of swine wastewater. Bioresour Technol 2020;300:122707.
[54] Nie E, He P, Zhang H, Hao L, Shao L, Lü F. How does temperature regulate anaerobic digestion? Renew Sustain Energy Rev 2021;150:111453.
[55] Doi AM, Stoskopf MK. The Kinetics of Oxytetracycline Degradation in Deionized Water under Varying Temperature, pH, Light, Substrate, and Organic Matter. J Aquat Anim Health 2000;12(3):246–53.
[56] Ratasuk N, Boonsaner M, Hawker DW. Effect of temperature, pH and illumination on abiotic degradation of oxytetracycline in sterilized swine manure. J Environ Sci Health A 2012;47(11):1687–94.
[57] Xuan R, Arisi L, Wang Q, Yates SR, Biswas KC. Hydrolysis and photolysis of oxytetracycline in aqueous solution. J Environ Sci Health B 2009;45(1):73–81.
[58] Feng D, Xia Ao, Liao Q, Nizami A-S, Sun C, Huang Y, et al. Carbon cloth facilitates semi-continuous anaerobic digestion of organic wastewater rich in volatile fatty acids from dark fermentation. Environ Pollut 2021;272:116030.
[59] Song C, Li W, Cai F, Liu G, Chen C. Anaerobic and microaerobic pretreatment for improving methane production from paper waste in anaerobic digestion. Front Microbiol 2021;12:688290.
[60] Zuo X, Yuan H, Wachemo AC, Wang X, Zhang L, Li J, et al. The relationships among sCOD, VFAs, microbial community, and biogas production during anaerobic digestion of rice straw pretreated with ammonia. Chin J Chem Eng 2020;28(1):286–92.
[61] Zhou JH, Shang XN, Wang ZW, Zhu CC, Wang SF. Effects of calcium concentration on up-flow multistage anaerobic reactor performance in treating bagasse spraying wastewater. BioResources 2019;14(2):4254–69.

[62] Conklin A, Stensel HD, Ferguson J. Growth kinetics and competition between *Methanosarcina* and *Methanosaeta* in mesophilic anaerobic digestion. Water Environ Res 2006;78(5):486–96.
[63] Lomans BP, Maas R, Luderer R, Op den Camp HJM, Pol A, van der Drift C, et al. Isolation and Characterization of *Methanomethylovorans* hollandica gen. nov., sp. nov., Isolated from Freshwater Sediment, a Methylotrophic Methanogen Able To Grow on Dimethyl Sulfide and Methanethiol. Appl Environ Microbiol 1999;65(8): 3641–50.
[64] Sakai S, Ehara M, Tseng I-C, Yamaguchi T, Bräuer SL, Cadillo-Quiroz H, et al. *Methanolinea mesophila* sp. nov., a hydrogenotrophic methanogen isolated from rice field soil, and proposal of the archaeal family *Methanoregulaceae* fam. nov. within the order *Methanomicrobiales*. International Journal of Systematic and Evolutionary Microbiology. 2012;62:1389-1395.
[65] Guo X, Sun C, Lin R, Xia Ao, Huang Y, Zhu X, et al. Effects of foam nickel supplementation on anaerobic digestion: Direct interspecies electron transfer. J Hazard Mater 2020;399:122830.
[66] Yamada T, Sekiguchi Y, Hanada S, Imachi H, Ohashi A, Harada H, et al. *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes *Anaerolineae* classis nov. and *Caldilineae* classis nov. in the bacterial *phylum Chloroflexi*. International Journal of Systematic and Evolutionary Microbiology. 2006;56(6):1331-1340.
[67] Zhou J, Holmes DE, Tang H-Y, Lovley DR. Correlation of key physiological properties of *Methanosarcina* isolates with environment of origin. Appl Environ Microbiol 2021;87(13). e00731-00721.
[68] Lee JTE, Lim EY, Zhang Le, Tsui T-H, Tian H, Yan M, et al. *Methanosarcina thermophila* bioaugmentation and its synergy with biochar growth support particles versus polypropylene microplastics in thermophilic food waste anaerobic digestion. Bioresour Technol 2022;360:127531.
[69] Deng Y, Zhang Yu, Gao Y, Li D, Liu R, Liu M, et al. Microbial community compositional analysis for series reactors treating high level antibiotic wastewater. Sci Total Environ 2012;46(2):795–801.