Microbial Community Evolution and Fate of Antibiotic Resistance Genes during Sludge Treatment in Two Full-Scale Anaerobic Digestion Plants with Thermal Hydrolysis Pretreatment

Tong, Juan; Fang, Ping; Zhang, Junya; Wei, Yuansong; Su, Yanyan; Zhang, Yifeng

Published in:
Bioresource Technology

Link to article, DOI:
[10.1016/j.biortech.2019.121575](https://doi.org/10.1016/j.biortech.2019.121575)

Publication date:
2019

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):

Tong, J., Fang, P., Zhang, J., Wei, Y., Su, Y., & Zhang, Y. (2019). Microbial Community Evolution and Fate of Antibiotic Resistance Genes during Sludge Treatment in Two Full-Scale Anaerobic Digestion Plants with Thermal Hydrolysis Pretreatment. *Bioresource Technology*, 288, [121575].
<https://doi.org/10.1016/j.biortech.2019.121575>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Accepted Manuscript

Microbial Community Evolution and Fate of Antibiotic Resistance Genes during Sludge Treatment in Two Full-Scale Anaerobic Digestion Plants with Thermal Hydrolysis Pretreatment

Juan Tong, Ping Fang, Junya Zhang, Yuansong Wei, Yanyan Su, Yifeng Zhang

PII: S0960-8524(19)30805-3
DOI: <https://doi.org/10.1016/j.biortech.2019.121575>
Article Number: 121575
Reference: BITE 121575

To appear in: *Bioresource Technology*

Received Date: 8 April 2019
Revised Date: 26 May 2019
Accepted Date: 27 May 2019

Please cite this article as: Tong, J., Fang, P., Zhang, J., Wei, Y., Su, Y., Zhang, Y., Microbial Community Evolution and Fate of Antibiotic Resistance Genes during Sludge Treatment in Two Full-Scale Anaerobic Digestion Plants with Thermal Hydrolysis Pretreatment, *Bioresource Technology* (2019), doi: <https://doi.org/10.1016/j.biortech.2019.121575>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Microbial Community Evolution and Fate of Antibiotic Resistance Genes during
Sludge Treatment in Two Full-Scale Anaerobic Digestion Plants with Thermal
Hydrolysis Pretreatment**

Juan Tong^{a,b,c} · Ping Fang^d · Junya Zhang^{a,b,c} · Yuansong Wei^{a,b,c,*} · Yanyan Su^e · Yifeng Zhang^f

^a State Key Joint Laboratory of Environmental Simulation and Pollution Control, Research
Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085,
China

^b Department of Water Pollution Control, Research Center for Eco-Environmental Sciences,
Chinese Academy of Sciences, Beijing 100085, China

^c University of Chinese Academy of Sciences, Beijing 100049, China

^d School of Environmental and Chemical Engineering, Xi'an Polytechnic University, Xi'an
710048, China

^e Carlsberg Research Laboratory, J.C. Jacobsens Gade 4, DK-1799 Copenhagen V,
Denmark

^f Department of Environmental Engineering, Technical University of Denmark, Copenhagen
Lyngby 2800, Denmark

Corresponding Author

Yuansong Wei*, Email: yswei@rcees.ac.cn

Abstract

Anaerobic digestion (AD) with thermal hydrolysis pretreatment is widely used as an efficient sludge treatment nowadays. However, the evolution of microbial community (especially for the archaea community), the fate of antibiotic resistance genes (ARGs), and their associations during such process in full-scale sludge treatment plants are rarely reported. Therefore, these scientific questions were explored at two full-scale sludge treatment plants through high-throughput sequencing and quantitative PCR. Results showed that *Methanobacterium* and *Methanosphaera* were the dominant archaea in thermal hydrolyzed sludge. The predominant bacteria in the sludge first shifted from nutrients removal functional bacteria to spore-forming bacteria after thermal hydrolysis, and then shifted to fermentative bacteria after AD. The full-scale plants could select *ermB*, *ermF*, *mefA/E*, *qnrS* and *tetM*. Though the bacteria and archaea biomass and community largely influenced the fate of ARGs, multiple linear regression analysis showed that the total ARGs were mainly affected by mobile genetic elements (MGEs).

Keywords Sludge thermal hydrolysis; Anaerobic digestion; Microbial community; Antibiotic resistance genes; Multiple linear regression analysis

1. Introduction

Advanced anaerobic digestion (AD) system with thermal hydrolysis pretreatment has been widely used for sludge treatment in recent years. One of the important reasons is that thermal pretreatment leads to the hydrolysis of sludge inducing the destruction of cell walls and the release of the cytoplasm contents, which can improve the substrate availability for

the methanogens growth and reduce the solid retention time of AD, and thereby boosting methane production (Xue et al., 2015). It is well known that thermal hydrolysis is suitable for waste materials which are high in carbohydrates and/or proteins (Barber 2016). Organic matters are released during thermal hydrolysis (Xue et al., 2015) and their degradation and methanogenic pathways during AD (Chen et al., 2018) have also been studied. Most of the studies have found that the optimal temperature to increase methane yield in the subsequent AD process ranges from 160 to 180 °C (Hii et al., 2014). The cooperation between microorganisms plays important roles in hydrolysis, fermentation, and acetogenesis, which are needed for biomethanation of organic matter (Yi et al., 2014). Therefore, the evolution of archaea and bacterial community during the thermal hydrolysis pretreatment and AD process is crucial to understand and optimize the systems. However, most of the studies are limited to lab-scale (Pei et al., 2014), and no full-scale investigation is available so far.

It is well known that wastewater treatment plants (WWTPs) are an important reservoir of antibiotic resistance bacteria (ARB) and associated antibiotic resistance genes (ARGs) (Pruden et al., 2013). The prevalence of ARGs during wastewater treatment in full-scale WWTPs has been well studied (Wei et al., 2018), while few studies are available on the fate of ARGs during advanced sludge anaerobic digestion in full-scale plants. For example, ever since the presence of the new type of carbapenem resistance gene (*bla_{NDM-1}*) was first reported in 2009 (Kumarasamy et al., 2010), it has been considered as a worldwide public health problem for its multi-drug resistance feature. Plasmids carrying *bla_{NDM-1}* can have up to 14 other antibiotic resistance determinants, resulting in multidrug-resistant or extreme

drug-resistant phenotypes. The prevalence of *bla*_{NDM-1} has been observed in wastewater treatment plants (Luo et al., 2014). However, the fate of *bla*_{NDM-1} during advanced sludge AD is still unknown. In addition, the mechanisms determining the fate of ARGs are rather complex and could be varied with the treatment technologies, and therefore it remains unclear which factors are the main drivers on the fate of ARGs (Manaia et al., 2018), especially in full-scale AD plants equipped with thermal hydrolysis pretreatment. Therefore, further study on the fate of ARGs and a better understanding of their main impact factors in the full-scale advanced AD treatment plant is very necessary and urgent.

In this study, for the first time, the evolution of archaea and bacterial community, the fate of ARGs, and their associations during sludge treatment in two full-scale AD plants integrated with thermal hydrolysis pretreatment were systematically explored. Specifically, the fate of ARGs including *bla*_{NDM-1} was studied and their influence factors were evaluated by multiple linear regression analyses. And then the uniform rules of the fate of ARGs during the two sludge treatment plants in the same city with a similar treatment process and the main driver on the fate of ARGs were evaluated. This study could provide a fundamental basis for a better understanding of the microbial community succession, the prevalence and the fates of ARGs during the advanced sludge treatment.

2. Methods and Materials

2.1 Samples collection

Two municipal wastewater treatment plants named Plant G and X in Beijing, China, both adopted Anaerobic-Anoxic-Oxic (A²/O) process for wastewater treatment, with treating

capacity of 1.0×10^6 and 6.4×10^5 m³/d, separately. The sludge line of these two municipal wastewater treatment plants both used advanced AD process with Cambi thermal hydrolysis pretreatment treating a mixture of waste activated sludge and primary sludge. The sludge treating capacity of plant G and X are 1358 m³/d and 900 m³/d with the water content of approx. 80%, respectively. Sludge consisting of 17% total solids (TS) were induced to thermal hydrolysis pretreatment, which includes hydrolysis at a temperature of 165 °C and pressure of 0.6 MPa for 30 min, followed by a sudden drop in pressure, resulting in so-called “steam explosion” tearing cells and fibers apart to further improve the disintegration effect. Subsequently, sludge consists of 8~12% TS were treated by the AD process running at 39 ~ 40°C with SRT of 18 days. The daily methane production performance of these two plants was not available.

Sludge samples were collected at the inlet (G1 and X1) and outlet (G2 and X2) of the thermal hydrolysis process, named as raw sludge and thermal hydrolyzed sludge, respectively. The samples of the outlet (G3 and X3) taken from the anaerobic digester was named here as digested sludge. Three independent sampling events of each plant were conducted from March to September in 2017.

2.2 Analytical methods

Certain characteristics, i.e. pH, alkalinity (ALK), soluble COD (sCOD), protein, polysaccharides, total ammonia nitrogen (TAN), ortho-phosphorus (OP) of the filtrates of each sludge sample were analyzed. The total solid (TS), volatile solid (VS), sCOD, ALK, ammonia nitrogen, and OP contents were measured according to the procedure of the

standard method (APHA, 2005). The pH was determined using a pH meter (pHs-3C, Leici, China). The protein and polysaccharides contents were measured as described in our previous study (Tong et al., 2018). Concentrations of volatile fatty acids (VFA), i.e. acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid, and n-valeric acid were determined by a gas chromatography (Shimadzu GC-2010 Plus, Japan) equipped with a flame ionization detector (GC-FID) and stabilwax-DA column (0.32 mm $\times$ 25 m $\times$ 0.25 μ m). Free ammonia nitrogen (FAN) was calculated as the following equation (Hansen et al., 1998).

$$[\text{FAN}] = [\text{TAN}] \left[1 + \frac{10^{-\text{pH}}}{10^{-(0.09018 + \frac{2729.92}{T})}} \right]^{-1} \quad (1)$$

Where [FAN] is the concentration of $[\text{NH}_3]$ (mg/L), [TAN] is the total ammonia nitrogen concentration (mg/L), and T (K) is the temperature.

2.3 DNA extraction and quantification

200 μ L of sludge samples were adopted for DNA extraction using the FastDNA Spin Kit for Soil (MP Biomedicals, US) according to the manufacturer's instructions. A NanoDrop 2000 (Thermo Scientific, USA) was used to determine DNA concentration and purity.

Bacterial and archaeal 16S rRNA gene copy numbers were quantified by quantitative PCR (q-PCR), and the functional gene *mcrA* involved in the methane production was also quantified.

2.4 High-throughput sequencing

The DNA extracts of triplicate samplings were combined for microbial community analysis. The primers 515F/806R were selected mainly for bacterial community analysis,

while the archaeal community was analyzed using the nested PCR by the primers Arch340F/Arch1000R and Arch349F/Arch806R. Sequencing was performed with Illumina high-throughput sequencing method. Pairs of reads from the original DNA fragments were merged using FLASH and then filtered using QIIME quality filters. PCR chimeras were filtered out in UCHIME. The screened high-quality reads were clustered by OTUs (Operational Taxonomic Units) at a 97% similarity level and the abundances and annotations of species were analyzed. The taxonomic classification of the sequences was performed using the Ribosomal Database Project (RDP) Classifier at the bootstrap cutoff of 80% suggested by the RDP. The sequences were uploaded to MG-RAST (<http://metagenomics.anl.gov>) under the project numbers (mgp88868).

2.5 Statistical analysis

The Chao1 index, Pielou index, and Shannon index were calculated by Mothur software (<http://www.mothur.org>) (Schloss et al., 2009). The heatmap illustrating the evolution of the bacterial community was conducted by Hengl 1.0 and clustered based on Euclidean distance. Network analysis was used to estimate the associations of archaea and bacterial community by Gephi 0.9.2 based on Spearman correlation analysis. Multiple linear regression analyses were applied to evaluate the importance of factors on the fate of ARGs (SPSS 20.0, IBM, USA) as described in our previous study (Tong et al., 2018). In brief, a dimension reduction for the dominant genera of bacteria and archaea was first conducted based on a PCA. The top master coordinates (coord1, coord2, coord3, etc.) with a cumulative variance proportion over 80% were adopted to express the microbial community (E-supplementary data for this

work can be found in e-version of this paper online). Based on F-tests, the least significant variables were removed from the model via a stepwise variable reduction. A p -value <0.05 indicates that the multiple linear regression model is statistically significant. The adjusted R^2 values of the linear regression models indicate the explanatory power of regression models that contain different numbers of predictors.

3. Results and discussion

3.1 Variations of sludge characteristics, *mcrA*, microbial biomass, and microbial diversity

Figure 1 summarizes the sludge characteristics, *mcrA*, and microbial biomass in different sludge samples. The raw sludge G1 and X1 had the same VS/TS ratio of 0.64. Compared to X1, the raw sludge G1 showed higher VS (23.5% higher), sCOD (143.9% higher), soluble protein (136.0% higher), soluble polysaccharides (154.3% higher), total VFA (237.6% higher), ammonia (416.2% higher), and OP (248.9% higher) content. Due to the longer storage time of raw sludge in plant G compared with that in plant X, fermentation proceeded in the storage container in plant G and resulted in the higher VFA in G1. After the thermal hydrolysis, the total VFA concentration decreased by 54.3% and 23.4% for plant G and X, respectively. However, the increase in organic matters after thermal hydrolysis of raw sludge X1 was higher than that of G1. For example, the sCOD, protein, and polysaccharides increased by 0.65, 4.45, 3.57 folds for sludge G1, but 2.77, 9.90, 5.16 folds for sludge X1. The possible reason for this phenomenon may be due to that the amount of the organic matters released was dependent on the organic compound composition of raw sludge and

the biodegradability of raw sludge (Hii et al., 2014). During the AD treatment, the increase of TAN from 485 ~ 518 to 1679 ~ 1879 mg/L was mainly due to the degradation of protein, which resulted in 273 and 142 mg/L FAN in the digested sludge of G3 and X3, respectively. Meanwhile, the increase of TAN also led the ALK to increase to 5681 and 6327 mg/L (i.e. 6.56- and 6.32-fold increase, respectively) in the digested sludge of G3 and X3.

The methyl coenzyme A gene (*mcrA*), encoding the α subunit of methyl coenzyme M reductase (the enzyme that catalyses the final step in methanogenesis), was used to monitor the activity of methanogenic biomass in anaerobic digesters (Morris et al., 2014). The copy number of *mcrA* decreased 2.05 and 1.07 logs after thermal hydrolysis compared with the raw sludge but increased 2.68 and 2.47 logs after AD compared with the thermal hydrolysed sludge for plant G and X, respectively. These results indicate that the thermal hydrolysis would also decrease the activity of methanogens along with the reduction of methanogenic biomass, while a reasonable increase of the activity of methanogens was found along with the proliferation of methanogenic biomass during the AD. The *mcrA* in digested sludge X3 was 0.31 log copies/g higher than that of G3, which indicated that the methanogens in X3 showed higher activity. This was consistent with the results that there were less residue sCOD (46.8% lower), total VFA (14.1% lower), acetic (8.6% lower) in the digested sludge X3 than that of G3, which means better utilization of these organic matters during AD.

Although the VS in the raw sludge G1 was 23.5% higher than that in X1, the copy number of archaea and bacteria were similar for the two raw sludge samples, with 9.40 (11.9) and 9.04 (11.7) log copies/g of archaea (bacteria) in G1 and X1, respectively. The

archaeal biomass decreased by 2.63 and 1.75 logs after thermal hydrolysis for plant G and X, respectively, and rebounded by 3.88 and 3.51 log after AD. Comparatively, only 1.30 and 0.72 log reduction of bacterial biomass was observed during thermal hydrolysis and 1.64 and 1.51 log increase during AD. These results indicate that archaea were more sensitive than bacteria during thermal hydrolysis treatment, while the AD process enhanced the proliferation of archaea.

The variations of archaeal and bacterial diversity were shown in Figure 2. The Chao1, Pielou, and Shannon index of bacteria reduced to 16.0% - 61.8% of that of the raw sludge after thermal hydrolysis, and then increased by 57.2% - 107.5% after AD. The same tendency of the three above-mentioned indexes was also observed for archaea diversity, with the only exception of the Pielou and Shannon index of archaea (decreased by 10.1% and 5.9% during the AD in plant G). Thus, the archaea diversity, especially its evenness in AD for plant G, did not increase, which might indicate that plant G did not reach the optimal capacity for methane production compared with plant X.

3.2 Evolution of archaeal community

In the raw sludge of G1 and X1, *Methanosaeta* (33.1% and 18.3%), *Methanospirillum* (28.6% and 36.4%), *Methanoregula* (8.6% and 16.0%), and *Methanomassiliicoccus* (4.4% and 13.0%) were the dominant archaea (Figure 3). After thermal hydrolysis, the abundance of *Methanosaeta* and *Methanospirillum* reduced by 8.1%~34.3% in both plants. However, *Methanosphaera* increased to 13.0% and 54.6%, and *Methanobacterium* increased to 14.2% and 20.0% in the thermal hydrolyzed sludge of G2 and X2, respectively. The temperature

range of *Methanospirillum* and *Methanosaeta* are 30 ~ 45°C (Garcia et al., 2006) and 10 ~ 70 °C, respectively (Kendall and Boone, 2006). Both of *Methanobacterium* and *Methanosphaera* belong to the order methanobacteriales, which can survive in a broader temperature range from 15 to 97 °C (Bonin and Boone, 2006). This is why these two genera archaea had higher survival ability than the other genera under the extreme condition at high temperature and pressure during thermal hydrolysis process.

After AD process, the genera *Methanoculleus* (24.0% and 34.6% in G3 and X3, respectively), *Methanospirillum* (33.5% and 29.2%), *Methanomassiliicoccus* (21.4% and 17.1%), *Methanosaeta* (13.2% and 10.7%) became the dominant archaea in the digested sludge. *Methanospirillum* and *Methanoculleus* are responsible for the hydrogenotrophic methanogenesis, and the latter is most commonly related to syntrophic acetate oxidation (Li et al., 2017). *Methanomassiliicoccus* can use methanol/methylamines (monomethylamine, dimethylamine, and trimethylamine) and H₂ for methane production (Kroninger et al., 2017) and be viewed as the mixture type of hydrogenotrophic and methylotrophic methanogens. *Methanosaeta* is acetoclastic methanogens with a higher affinity for acetate but lower growth rate compared with *Methanosarcina* (Jetten et al., 1992).

The abundance of acetoclastic methanogens (*Methanosaeta* and *Methanosarcina*) accounted for 38.16% and 19.75% in the raw sludge of G1 and X1, respectively. The abundance reduced to 15.61% and 13.59% after thermal hydrolysis, and then slightly increased to 16.71% and 13.69% in the digested sludge. Comparatively, the hydrogenotrophic methanogens accounted for 49.94% and 60.49% in the raw sludge of G1

and X1, which accounted for 64.13% and 25.49% in the thermal hydrolyzed sludge, and 60.93% and 66.61% for the digested sludge. These results indicated that hydrogenotrophic methanogens were the dominant methanogens in the raw sludge and digested sludge, and hydrogenotrophic pathway was the main biochemical pathway of methanogenesis in the AD system. In general, acetoclastic methanogens are more sensitive to ammonia stress than the hydrogenotrophic methanogens (Zhang et al., 2014). Angenent et al., (2002) reported that *Methanosarcina* decreased from 3.8% to 1.2% as the TAN increased from 2.0 to 3.6 g/L, while hydrogen-utilizing methanogens increased from 2.3% to 7.0%. Nevertheless, it has also been found that *Methanosarcina* were dominant in a mesophilic AD system for waste sludge with TAN of 2.9 ~ 3.3 g/L in the digesters (Liu et al., 2016), which was higher than the TAN concentration (1.7 ~ 1.9 g/L) in the present study. It has been suggested that no general rules could be determined about which process parameters favour the pathway (Demirel and Scherer, 2008). Therefore, further studies on the pathway during the AD with thermal hydrolysis pretreatment are needed.

3.3 Evolution of bacterial community

The dominant bacteria in raw sludge of G1 and X1 were *Nitrospira* (3.63% and 2.50%), *Dechloromonas* (2.37% and 4.38%), *Dokdonella* (2.20% and 2.82%), *Comamonas* (2.06% and 2.33%), *Thauera* (1.69% and 3.26%) and *Zoogloea* (1.62% and 6.36%), etc (Figure 4). *Nitrospira* were nitrite oxidation bacteria (NOB) which were prevalent in wastewater treatment systems (Xu et al., 2018). *Dechloromonas* and *Thauera* were typical denitrifying bacteria in the wastewater treatment process (Wu et al., 2013). Both of these two genera

exhibit a strong ability to accumulate polyphosphate within cells and play a key role in efficient phosphorous removal during wastewater treatment process (Yun et al., 2018). It has been reported that *Comamonas* can also utilize diverse carbon sources in wastewater treatment as denitrifying bacteria (Etchebehere et al., 2001). These results suggest that the predominant bacteria in the raw sludge were responsible for nutrients removal.

There was an evident shift of bacterial community after thermal hydrolysis. The dominant genera were *Clostridium sensu stricto* (51.3%), *Thermobrachium* (17.5%), *Bacillus* (8.41%), *Falsibacillus* (5.84%), and *Thermoanaerobacterium* (4.62%) in the thermal hydrolyzed sludge of G2. *Clostridium sensu stricto* (41.3%), *Clostridium XI* (34.6%), *Kurthia* (12.0%), *Caloramator* (3.98%), *Enterococcus* (3.39%) were the top 5 genera in the thermal hydrolyzed sludge of X2. All the above dominant genera belong to phylum Firmicutes. The augmentation of Firmicutes after thermal hydrolysis pretreatment was consistent with the previous study in a lab-scale research (Tong et al., 2018). Members of the genus *Clostridium sensu stricto* are anaerobic, acid-tolerant, spore-forming bacterium with good thermostability and drying resistance (Wiegel et al., 2006), which featured the abundance of *Clostridium sensu stricto* after thermal hydrolysis. The promotion of the members of order Clostridiales after thermal hydrolyses, such as *Clostridium sensu stricto*, *Clostridium XI*, *Caloramator*, and *Thermobrachium*, was in consistent with a previous study that the order Clostridiales was selectively accumulated during the thermal hydrolysis pretreatment (Chen et al., 2018). In addition, it has been reported that *Caloramator*, *Thermobrachium*, and *Thermoanaerobacterium* were thermophilic anaerobes, and the first

two genera are also alkaliphilic anaerobes (Ollivier et al., 2000). *Falsibacillus* are spore-forming bacteria (Zhou et al., 2009), whereas *Bacillus* can produce endospores (Baruzzi et al., 2011). These properties help these bacteria survive through the thermal hydrolysis process. *Thermoanaerobacterium* is a saccharolytic heterotrophs and can use thiosulfate as an electron acceptor (Ollivier et al., 2000). Furthermore, many species of *Bacillus* are able to produce copious amounts of enzymes, such as amylases, lipases, and proteases (Baruzzi et al., 2011). These results indicate that the thermal hydrolyzed sludge was dominant by spore-forming bacteria belonging to the class of Clostridia and Bacilli, which classes belong to phylum Firmicutes. The predominant bacteria contribute to methane production during the AD process.

As for the digested sludge of G3 and X3, *Smithella* (7.90% and 8.52%), *Petrimonas* (7.50% and 5.84%), *Saccharicrinis* (6.97% and 5.05%), *Syntrophomonas* (5.50% and 4.39%) and *Ercella* (4.51% and 3.39%) were the dominant bacteria. *Smithella* is syntrophic propionate-oxidizing bacteria, which grows syntrophically on propionate only with methanogenic bacteria to remove H₂ and formate from the system (Liu et al., 1999). *Petrimonas* are fermenters of carbohydrates, certain organic acids (e.g., acetic acid, propionic acid, isovaleric acid), CO₂ and traces of H₂ (Grabowski et al., 2005). A member of *Petrimonas*, i.e. *P. sulfuriphila* can use both nitrate and elemental sulfur as electron acceptors and reduce them to ammonium and sulfide, respectively (Grabowski et al., 2005). *Saccharicrinis* also ferments carbohydrates (Yang et al., 2014). *Syntrophomonas* are butyrate-utilizing acetogens, the member of which can degrade long-chain fatty acids

(LCFAs) (Sousa et al., 2007). The above results indicate that the digested sludge was dominant by fermentative bacteria which could provide acetate and H_2 for methanogens to further produce methane.

3.4 Associations of archaea and bacterial community

It has been suggested that the cooperation of microorganisms plays respective roles in hydrolysis, fermentation, and acetogenesis during mineralization of organic matter to biogas (Yi et al., 2014). Therefore, the network analysis was used to evaluate the associations of archaea and bacteria during the thermal hydrolysis and AD treatment (Figure 5). The genera of bacteria *Ferribacterium*, *Macellibacteroides*, *Kurthia*, and *Phaeodactylibacter*, etc. showed a positive effect on 4 genera of methanogens (*methanomethylovorans*, *methanolobus*, *methanoregula*, *methanosphaerula*, and *methanosaeta*, etc.), which indicated that these bacteria might play an important role in methane production. For example, *Ferribacterium* can promote the methanogenesis by oxidizing organic acids with Fe (III) as an electron acceptor (Cummings et al., 1999). It has been reported that the biological reduction of Fe (III) could lower the reduction potential of the system and enhance methanogenesis (Zhang et al., 2013). *Macellibacteroides* can degrade carbohydrates and the main fermentation products from glucose metabolism are normally lactate, acetate, butyrate and isobutyrate (Jabari et al., 2012). Therefore, the positive associations between the above bacteria and methanogens were observed in this study. However, *Desulfotomaculum* showed a significant negative effect on *Methanolobus*, *Methanoregula*, *Methanosphaerula*. This could be due to that *Desulfotomaculum* is sulfate-reducing bacteria and could compete

for acetate with methanogens (Raskin et al., 1996). These results indicate that *Methanolobus*, *Methanoregula*, *Methanosphaerula* might be more sensitive to sulfates or sulfides than other methanogens. Furthermore, there were both competitions of substrate for methane production, and syntrophic cooperation between different methanogens.

3.5 Fate of ARGs and MGEs

The total absolute abundance of ARGs was 115.7 and 113.1 log copies/g in the raw sludge of plant G and X, with 6.58 ~ 10.16 log copies/g for individual ARGs. After the thermal hydrolysis of plant G and X, the total absolute ARGs was reduced by 25.5 and 20.8 logs, with 4.71 and 3.55 logs reduction for total MGEs (Figure 6a and 6b). These results were inconsistent with the previous lab-scale studies that thermal hydrolysis leads to a significant decrease in ARGs and MGEs (Pei et al., 2016). During thermal hydrolysis, the hardest removal ARG was *bla_{CTX-M}*, with 0.17 and 0.13 log reduction in plant G and X, respectively, followed by *mefA/E*, with 1.03 and 0.19 log reduction. The total ARGs and MGEs rebounded after AD, which was in agreement with the previous lab-scale study (Pei et al., 2016). Compared with the raw sludge, the total MGEs reduced by 5.7% and 0.8% (i.e. 1.05 and 0.16 log reduction) in G2 and X2, while the total absolute abundance of ARGs increased by 0.4% and 5.0% (i.e. 0.41 and 5.93 log increase).

The copy numbers of *bla_{NDM-1}* in the raw sludge were 7.22 and 7.02 log copies/g for plant G and X, which was close to the concentration of *bla_{NDM-1}* in dewatered sludge from two WWTPs in northern China (Luo et al., 2014). Although the thermal hydrolysis could reduce 1.05-1.40 logs of *bla_{NDM-1}*, the copy numbers of *bla_{NDM-1}* increased significantly after AD

and resulted in even 0.57-1.53 logs higher than that in the raw sludge. These results indicate that the advanced AD process might promote the multi-resistance potential of digested sludge. Overall, the thermal hydrolysis and AD process reduced the copy numbers of *bla*_{TEM}, *tetA*, *tetX*, *suII*, *intI1*, but induced the proliferation of *bla*_{CTX-M}, *ermB*, *ermF*, *mefA/E*, *qnrS*, *tetM* in the AD digested sludge. The effective reduction of *tetX* during the sludge pretreatment and subsequent AD was consistent with results of our previous study (Tong et al., 2016), in which the fate of ARGs in the sewage sludge was investigated during the AD with microwave pretreatment. These results suggest *tetX* tended to be removed during the advanced AD of sewage sludge. However, there was one order of magnitude of copy numbers of total ARGs in the digested sludge higher than that in the raw sludge of plant G and X. The reason was that the AD with thermal hydrolysis pretreatment selected some ARGs, such as *ermB*, *ermF*, *mefA/E*, *qnrS* and *tetM*, and resulted in higher copy numbers of total ARGs detected in this study in the digested sludge than that in the raw sludge.

Similar to the fate of the absolute abundance of total ARGs and MGEs, their total relative abundances were reduced during the thermal hydrolysis but increased after AD (Figure 6c). The most prevalent ARGs in the raw sludge were *suII* and *suIII*. After thermal hydrolysis, the relative abundance of most ARGs and MGEs reduced with the exception of the relative abundance of *mefA/E*. The decrease in the relative abundance of MGEs indicates that the horizontal gene transfer (HGT) reduced during thermal hydrolysis. The increase in the relative abundance of *mefA/E* might be mainly due to the proliferation of the host bacteria, such as *Clostridium* and *Enterococcus* according to the antibiotic resistance gene database

(ARDB, <http://ardb.cbcb.umd.edu/>) after thermal hydrolysis, resulting in a higher possibility of vertical and horizontal gene transfer for *mefA/E*.

Furthermore, the relative abundance of *ermB*, *ermF*, and *qnrS* increased after AD and became the most prevalent ARGs in the digested sludge of both G3 and X3, which indicated that these genes tend to be persistent ARGs in sludge compared with other ARGs observed in this study. In our previous study, the relative abundance of *qnrS* increased in all the effluents regardless of the wastewater treatment technology used in WWTPs (Tong et al., 2019). It has been found that *qnr* genes associated with the genes encoding for extended spectrum β -lactamases and *ampC* β -lactamases (Strahilevitz et al., 2009). The associations of *qnrS* with other resistance determinants might promote the dissemination and maintenance of *qnrS*, as the additional resistance genes would allow co-selection of *qnrS* (Hernández et al., 2011). Likewise, *ermB* and *ermF* are often linked with *tetM* and *tetQ*, respectively (Roberts et al., 1999). Furthermore, it has been widely accepted that *qnrS*, *ermB*, and *ermF* were associated with transposons (Hernández et al., 2011; Roberts et al., 1999), which could promote the dissemination and maintenance for these genes.

3.6 Factors affecting the fate of ARGs

Stepwise multiple linear regression analyses were applied to evaluate the importance of sludge characteristics (pH, ALK, sCOD, protein, polysaccharides, VFA, ammonia, and OP), archaea and bacterial biomass, archaea and bacterial community composition (in the genus level after dimension reduction), the diversity of archaea and bacterial (Chao1, Pielou, and Shannon index), and MGEs (*intI1* and *Tn916/1545*) on the fate of individual ARGs and total

ARGs during sludge treatment (in Table 1). The multiple linear regression models fit the data well ($p < 0.02$) and could explain 72.4% - 99.8% of the variations in individual ARGs and total ARGs.

Multiple linear regression results showed that MGEs was the main factor impact on the fate of total ARGs during sludge treatment. Our previous study also concluded that MGEs were the main drivers for pharmaceutical sludge treatment by AD with different pretreatments (Tong et al., 2018). These results suggest that the total ARGs might be mainly driven by MGEs during the advanced AD process regardless of the sludge type and the configuration of the reactors. However, compared with the municipal sludge in the present study, different major influential factors on the fate of the individual ARGs were found for the treatment of pharmaceutical sludge by AD with different pretreatments (Tong et al., 2018). For example, the major factor is bacteria biomass and *intI1* for the fate of *ermB* in municipal sludge and in pharmaceutical sludge, respectively. These results indicate that different sludge source, reactors and process parameters could influence the main drivers of individual ARGs due to the complicated interaction of the influence factors.

Models show that sludge characteristics, such as ammonia, protein and polysaccharides, significantly influenced the fate of ARGs including *bla*_{CTX-M}, *sul I*, and *qnrA*. In addition, Pielou, Shannon, and Chao1 index of archaea and bacteria showed a positive impact on the ARGs, which confirmed that richer microbial diversity might lead to relatively wider ARGs host range and then result in an increase in ARGs (Ma et al., 2011). Furthermore, the bacteria biomass and the community composition played an important role in the fate of

ermB, *tetM* and *bla*_{NDM-1}. In addition, it is notable that the archaea biomass and archaeal community could also significantly affect the fate of ARGs, such as *bla*_{NDM-1}, *ermF*, *mefA/E*, *qnrS*, and *sulIII* during the advanced AD of sludge treatment. Bacterial community were viewed as important factors to the fate of ARGs in many studies (Chen et al., 2016; Zheng et al., 2017), while the influence of archaea biomass and archaeal community on ARGs was rarely mentioned. It has been reported that some archaea are the potential ARGs host. For example, *Methanobrevibacter*, a typical methanogenic archaea genus, mainly carries *tet32*, *ermB* and aminoglycoside phosphotransferase (Li et al., 2015). In addition, horizontal gene transfer is common in many bacteria and archaea through transformation, conjugation and transduction (Palmer and Kishony, 2013). Therefore, it is reasonable that archaea biomass and archaeal community could influence the fate of ARGs during sludge treatment.

4. Conclusions

During thermal hydrolysis and AD treatment in full-scale plants, the predominant bacteria shifted from nutrients removal functional bacteria to spore-forming bacteria after thermal hydrolysis, and then to fermentative bacteria in the digested sludge. Archaea *Methanobacterium* might compete with *Methanospirillum* and *Methanomassiliicoccus*, but cooperate with *Methanosphaera* and *Methanothermobacter*. *tetX* tended to be removed, while *ermB*, *ermF*, *mefA/E*, *qnrS* and *tetM* were selected. In addition to the bacteria, archaea biomass and community also presented important impacts on the fate of ARGs. MGEs might be the main influential factor for the total ARGs during the advanced AD of sludge.

E-supplementary data for this work can be found in e-version of this paper online.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 51578535).

References

1. Barber, W.P.F., 2016. Thermal hydrolysis for sewage treatment: A critical review. *Water Res.* 104, 53-71.
2. Baruzzi, F., Quintieri, L., Morea, M. and Caputo, L., 2011. Antimicrobial compounds produced by *bacillus* spp. and applications in food. *Science against microbial pathogens: communicating current research and technological advances* 2, 1102-1111.
3. Bonin A.S., Boone D.R. 2006. The Order Methanobacteriales. In: Dworkin M., Falkow S., Rosenberg E., Schleifer KH., Stackebrandt E. (EDS) *The Prokaryotes*. Springer, New York, NY.
4. Chen, Q., An, X., Li, H., Su, J., Ma, Y. and Zhu, Y.G., 2016. Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. *Environ. Int.* 92-93, 1-10.
5. Chen, S., Li, N., Dong, B., Zhao, W., Dai, L. and Dai, X., 2018. New insights into the enhanced performance of high solid anaerobic digestion with dewatered sludge by thermal hydrolysis: Organic matter degradation and methanogenic pathways. *J. Hazard. Mater.* 342, 1-9.
6. Cummings, D.E., Caccavo Jr, F., Spring, S. and Rosenzweig, R.F., 1999.

- Ferribacterium limneticum*, gen. nov., sp. nov., an Fe (III)-reducing microorganism isolated from mining-impacted freshwater lake sediments. Arch. Microbiol. 171(3), 183-188.
7. Demirel, B. and Scherer, P., 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: A review. Reviews in Environmental Science and Bio/Technology 7(2), 173-190.
  8. Etchebehere, C., Errazquin, M.I., Dabert, P., Moletta, R. and Muxi, L., 2001. *Comamonas nitrativorans* sp. nov., a novel denitrifier isolated from a denitrifying reactor treating landfill leachate. Int. J. Syst. Evol. Microbiol. 51(Pt 3), 977-983.
  9. Garcia, J.L., Ollivier, B. and Whitman, W.B. (2006) The prokaryotes, pp. 208-230, Springer.
  10. Grabowski, A., Tindall, B.J., Bardin, V., Blanchet, D. and Jeanthon, C., 2005. *Petrimonas sulfuriphila* gen. Nov., sp. Nov., a mesophilic fermentative bacterium isolated from a biodegraded oil reservoir. Int. J. Syst. Evol. Microbiol. 55(3), 1113-1121.
  11. Hansen, K.H., Angelidaki, I. and Ahring, B.K., 1998. Anaerobic digestion of swine manure: Inhibition by ammonia. Water Res. 32(1), 5-12.
  12. Hernández, A., Sanchez, M.B. and Martínez, J.L., 2011. Quinolone resistance: Much more than predicted. Front. Microbiol. 2, 22.
  13. Hii, K., Baroutian, S., Parthasarathy, R., Gapes, D.J. and Eshtiaghi, N., 2014. A review of wet air oxidation and thermal hydrolysis technologies in sludge treatment. Bioresour.

- Technol. 155, 289-299.
14. Jabari, L., Gannoun, H., Cayol, J.-L., Hedi, A., Sakamoto, M., Falsen, E., Ohkuma, M., Hamdi, M., Fauque, G. and Ollivier, B., 2012. *Macellibacteroides fermentans* gen. nov., sp. nov., a member of the family porphyromonadaceae isolated from an upflow anaerobic filter treating abattoir wastewaters. Int. J. Syst. Evol. Microbiol. 62(10), 2522-2527.
  15. Jetten, M.S., Stams, A.J. and Zehnder, A.J., 1992. Methanogenesis from acetate: A comparison of the acetate metabolism in *methanothrix soehngenii* and *methanosarcina* spp. FEMS Microbiol. Lett. 88(3-4), 181-197.
  16. Kendall, M.M. and Boone, D.R. (2006) The prokaryotes, pp. 244-256, Springer.
  17. Kroninger, L., Gottschling, J. and Deppenmeier, U., 2017. Growth characteristics of *methanomassiliicoccus luminyensis* and expression of methyltransferase encoding genes. Archaea 2017, 2756573.
  18. Kumarasamy, K.K., Toleman, M.A., Walsh, T.R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C.G., Irfan, S., Krishnan, P., Kumar, A.V., Maharjan, S., Mushtaq, S., Noorie, T., Paterson, D.L., Pearson, A., Perry, C., Pike, R., Rao, B., Ray, U., Sarma, J.B., Sharma, M., Sheridan, E., Thirunarayan, M.A., Turton, J., Upadhyay, S., Warner, M., Welfare, W., Livermore, D.M. and Woodford, N., 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. Lancet Infect. Dis. 10(9), 597-602.
  19. Li, B., Yang, Y., Ma, L.P., Ju, F., Guo, F., Tiedje, J.M. and Zhang, T., 2015.

- Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *Isme Journal* 9(11), 2490-2502.
20. Li, N., Xue, Y., Chen, S., Takahashi, J., Dai, L. and Dai, X., 2017. Methanogenic population dynamics regulated by bacterial community responses to protein-rich organic wastes in a high solid anaerobic digester. *Chem. Eng. J.* 317, 444-453.
  21. Liu, C., Li, H., Zhang, Y., Si, D. and Chen, Q., 2016. Evolution of microbial community along with increasing solid concentration during high-solids anaerobic digestion of sewage sludge. *Bioresour. Technol.* 216, 87-94.
  22. Liu, Y., Balkwill, D.L., Aldrich, H.C., Drake, G.R. and Boone, D.R., 1999. Characterization of the anaerobic propionate-degrading syntrophs *smithella propionica* gen. nov., sp. nov. and *syntrophobacter wolinii*. *Int. J. Syst. Evol. Microbiol.* 49(2), 545-556.
  23. Luo, Y., Yang, F., Mathieu, J., Mao, D., Wang, Q. and Alvarez, P.J.J., 2014. Proliferation of multidrug-resistant new delhi metallo- β -lactamase genes in municipal wastewater treatment plants in Northern China. *Environ. Sci. Tech. Let.* 1(1), 26-30.
  24. Ma, Y., Wilson, C.A., Novak, J.T., Riffat, R., Aynur, S., Murthy, S. and Pruden, A., 2011. Effect of various sludge digestion conditions on sulfonamide, macrolide, and tetracycline resistance genes and class 1 integrons. *Environ. Sci. Technol.* 45(18), 7855-7861.
  25. Manaia, C.M., Rocha, J., Scaccia, N., Marano, R., Radu, E., Biancullo, F., Cerqueira, F., Fortunato, G., Iakovides, I.C., Zammit, I., Kampouris, I., Vaz-Moreira, I. and Nunes,

- O.C., 2018. Antibiotic resistance in wastewater treatment plants: Tackling the black box. Environ. Int. 115, 312-324.
26. Morris, R., Schauer-Gimenez, A., Bhattad, U., Kearney, C., Struble, C.A., Zitomer, D. and Maki, J.S., 2014. Methyl coenzyme M reductase (*mcrA*) gene abundance correlates with activity measurements of methanogenic H₂ /CO₂ -enriched anaerobic biomass. Microb. Biotechnol. 7(1), 77-84.
27. Ollivier, B., Patel, B. and Garcia, J.L. (2000) Journey to diverse microbial worlds, pp. 73-90, Springer.
28. Palmer, A.C. and Kishony, R., 2013. Understanding, predicting and manipulating the genotypic evolution of antibiotic resistance. Nature Reviews Genetics 14(4), 243.
29. Pei, J., Yao, H., Wang, H., Ren, J. and Yu, X., 2016. Comparison of ozone and thermal hydrolysis combined with anaerobic digestion for municipal and pharmaceutical waste sludge with tetracycline resistance genes. Water Res. 99, 122-128.
30. Pruden, A., Larsson, D.G., Amezquita, A., Collignon, P., Brandt, K.K., Graham, D.W., Lazorchak, J.M., Suzuki, S., Silley, P., Snape, J.R., Topp, E., Zhang, T. and Zhu, Y.G., 2013. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. Environ. Health Perspect. 121(8), 878-885.
31. Raskin, L., Rittmann, B.E. and Stahl, D.A., 1996. Competition and coexistence of sulfate-reducing and methanogenic populations in anaerobic biofilms. Appl. Environ. Microb. 62(10), 3847-3857.
32. Roberts, M.C., Sutcliffe, J., Courvalin, P., Jensen, L.B., Rood, J. and Seppala, H., 1999.

- Nomenclature for macrolide and macrolide-lincosamide-streptogramin b resistance determinants. *Antimicrob. Agents Chemother.* 43(12), 2823-2830.
33. Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., ... & Weber, C. F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microb.* 75(23), 7537-7541.
  34. Sousa, D.Z., Smidt, H., Alves, M.M. and Stams, A.J., 2007. *Syntrophomonas zehnderi* sp. nov., an anaerobe that degrades long-chain fatty acids in co-culture with *methanobacterium formicicum*. *Int. J. Syst. Evol. Microbiol.* 57(3), 609-615.
  35. Strahilevitz, J., Jacoby, G.A., Hooper, D.C. and Robicsek, A., 2009. Plasmid-mediated quinolone resistance: A multifaceted threat. *Clin. Microbiol. Rev.* 22(4), 664-689.
  36. Tong, J., Liu, J., Zheng, X., Zhang, J., Ni, X., Chen, M. and Wei, Y., 2016. Fate of antibiotic resistance bacteria and genes during enhanced anaerobic digestion of sewage sludge by microwave pretreatment. *Bioresour. Technol.* 217, 37-43.
  37. Tong, J., Lu, X., Zhang, J., Angelidaki, I. and Wei, Y., 2018. Factors influencing the fate of antibiotic resistance genes during thermochemical pretreatment and anaerobic digestion of pharmaceutical waste sludge. *Environ. Pollut.* 243, 1403-1413.
  38. Tong, J., Tang, A., Wang, H., Liu, X., Huang, Z., Wang, Z., Zhang, J., Wei, Y., Su, Y. and Zhang, Y., 2019. Microbial community evolution and fate of antibiotic resistance genes along six different full-scale municipal wastewater treatment processes. *Bioresour. Technol.* 272, 489-500.

39. Wei, Z., Feng, K., Li, S., Zhang, Y., Chen, H., Yin, H., Xu, M. and Deng, Y., 2018.
Exploring abundance, diversity and variation of a widespread antibiotic resistance gene
in wastewater treatment plants. *Environ. Int.* 117, 186-195.
40. Wiegel, J., Tanner, R. and Rainey, F.A. (2006) *The prokaryotes*, pp. 654-678, Springer.
41. Wu, W., Yang, L. and Wang, J., 2013. Denitrification performance and microbial
diversity in a packed-bed bioreactor using PCL as carbon source and biofilm carrier.
Appl. Microbiol. Biotechnol. 97(6), 2725-2733.
42. Xu, J., He, J., Wang, M. and Li, L., 2018. Cultivation and stable operation of aerobic
granular sludge at low temperature by sieving out the batt-like sludge. *Chemosphere*
(211), 1219-1227.
43. Xue, Y., Liu, H., Chen, S., Dichtl, N., Dai, X. and Li, N., 2015. Effects of thermal
hydrolysis on organic matter solubilization and anaerobic digestion of high solid sludge.
Chem. Eng. J. 264, 174-180.
44. Yang, S.H., Seo, H.S., Woo, J.H., Oh, H.M., Jang, H., Lee, J.H., Kim, S.J. and Kwon,
K.K., 2014. *Carboxylicivirga* gen. Nov. In the family *marinilabiliaceae* with two novel
species, *carboxylicivirga mesophila* sp. nov. and *carboxylicivirga taeansensis* sp. nov.,
and reclassification of *cytophaga fermentans* as *saccharicrinis fermentans* gen. nov.,
comb. nov. *Int. J. Syst. Evol. Microbiol.* 64(4), 1351-1358.
45. Yi, J., Dong, B., Jin, J. and Dai, X., 2014. Effect of increasing total solids contents on
anaerobic digestion of food waste under mesophilic conditions: Performance and
microbial characteristics analysis. *PLoS One* 9(7), e102548.

46. Yun, G., Lee, H., Hong, Y., Kim, S., Daigger, G.T. and Yun, Z., 2018. The difference of morphological characteristics and population structure in PAO and DPAO granular sludges. *J. Environ. Sci-China*. 76, 388-402.
47. Zhang, C., Yuan, Q. and Lu, Y.H., 2014. Inhibitory effects of ammonia on methanogen *mcrA* transcripts in anaerobic digester sludge. *FEMS Microbiol. Ecol.* 87(2), 368-377.
48. Zhang, J., Dong, H., Liu, D. and Agrawal, A., 2013. Microbial reduction of Fe(III) in smectite minerals by thermophilic methanogen *methanothermobacter thermautotrophicus*. *Geochim. Cosmochim. Acta* 106, 203-215.
49. Zheng, J., Gao, R.X., Wei, Y.Y., Chen, T., Fan, J.Q., Zhou, Z.C., Makimilua, T.B., Jiao, Y.N. and Chen, H., 2017. High-throughput profiling and analysis of antibiotic resistance genes in east Tiaoxi river, China. *Environ. Pollut.* 230, 648-654.
50. Zhou, Y., Xu, J., Xu, L. and Tindall, B.J., 2009. *Falsibacillus pallidus* to replace the homonym *bacillus pallidus* Zhou *et al.* 2008. *Int. J. Syst. Evol. Microbiol.* 59(12), 3176-3180.

Figure lists

Figure 1 Variations of sludge characteristics.

Figure 2 Variation of community diversity of (a) Archaeal and (b) bacterial during sludge treatment.

Figure 3 Archaeal community evolution in the genus level during sludge treatment.

Figure 4 Heat map of bacterial community in the genus level during sludge treatment

(based on the genus abundance top 10 genera in log 10 transformation).

Figure 5 Associations between the archaea and bacterial community by network analysis.

Blue nodes: methanogens; yellow nodes: other archaea; orange nodes: dominant bacteria (top 10 genera of each sample). Pink lines: positive correlation; Green lines: negative correlation.

Figure 6 Variations of the absolute and relative abundance of ARGs during sludge

treatment. (a) The removed Absolute abundance of ARGs and MGEs in plant D; (b)

Removed Absolute abundance of ARGs and MGEs in plant X; (c) Relative abundance of ARGs and MGEs.

Figure 1 Variations of sludge characteristics.

Figure 2 Variation of community diversity of (a) Archaeal and (b) bacterial during sludge treatment.

Figure 3 Archaeal community evolution in the genus level during sludge treatment.

Figure 4 Heat map of bacterial community in the genus level during sludge treatment

(based on the genus abundance top 10 genera in log 10 transformation).

Figure 5 Associations between the archaea and bacterial community by network analysis.

Blue nodes: methanogens; yellow nodes: other archaea; orange nodes: dominant bacteria

(top 10 genera of each sample). Pink lines: positive correlation; Green lines: negative

correlation.

Figure 6 Variations of the absolute and relative abundance of ARGs during sludge treatment. (a) The removed Absolute abundance of ARGs and MGEs in plant D; (b) Removed Absolute abundance of ARGs and MGEs in plant X; (c) Relative abundance of ARGs and MGEs.

Table 1 Multiple linear regression models for ARGs.

Absolute abundance of ARG (log copies • g ⁻¹)	Multiple linear regression models	<i>p</i> -Value ^a	Interpretation of models (%)	Inter
<i>bla</i> _{TEM}	<i>bla</i> _{TEM} = 6.586 + 4.81×10 ⁻⁴ (Chao1) _B	0.001	92.4	(Chao1) _B
<i>bla</i> _{CTX-M}	<i>bla</i> _{CTX-M} = 6.493 + 3.24 ×10 ⁻⁴ Ammonia	0.020	72.4	Ammonia
<i>bla</i> _{NDM-1}	<i>bla</i> _{NDM-1} = 0.687 + 0.704 Archaea + 0.364 (Coord3) _B	0.004	95.7	Archaea Archaea
<i>ermB</i>	<i>ermB</i> = -17.195 + 2.227 Bacteria	0.000	97.4	Bacteria
<i>ermF</i>	<i>ermF</i> = 0.026 + 0.961 Archaea + 1.59×10 ⁻⁴ (Chao1) _A	0.000	99.7	Archaea
<i>mefA/E</i>	<i>mefA/E</i> = 5.723 + 0.417 Archaea - 0.235 (Coord3) _A	0.000	99.0	Archaea Archaea
<i>qnrA</i>	<i>qnrA</i> = 6.880 - 0.001 Polysaccharides + 0.283 (Shannon) _A	0.000	99.4	Polysaccharides Polysaccharides
<i>qnrS</i>	<i>qnrS</i> = 1.149 + 0.737 Archaea + 3.42×10 ⁻⁴ (Chao1) _A	0.001	98.5	Archaea Archaea
<i>tetA</i>	<i>tetA</i> = 1.026 + 0.739 <i>intI1</i>	0.000	97.1	<i>intI1</i>
<i>tetX</i>	<i>tetX</i> = 5.078 + 0.688 (Shannon) _B	0.000	99.8	(Shannon) _B
<i>tetM</i>	<i>tetM</i> = -3.872 + 0.728 Bacteria + 0.471 Tn916/1545	0.000	99.7	Bacteria Bacteria
<i>sulI</i>	<i>sulI</i> = 10.337 - 3.67×10 ⁻⁴ Protein	0.003	88.6	Protein
<i>sulII</i>	<i>sulII</i> = 5.510 + 6.062 (Pielou) _B + 0.217 (Coord2) _A	0.000	99.8	(Pielou) _B (Pielou) _B
Total ARGs	Total ARGs = 7.859 + 11.645 Tn916/1545 + 2.726 (Coord2) _A	0.000	99.2	Tn916/1545 Tn916/1545

Notes: (Chao1)_B and (Chao1)_A---represents Chao1 index of bacteria and archaea, respectively; (Shannon)_B and (Pielou)_B ---represents Shannon and Pielou index of bacteria, respectively; (Coord1)_B and (Coord1)_A ---represents Coord1 of bacteria and archaea, respectively; archaea and bacteria---represents archaea and bacteria biomass, respectively.

Highlights

- Dominant bacteria shifted from nutrients removal bacteria to fermentative bacteria.
- Hydrogenotrophic pathway is the main biochemical pathway of methanogenesis in AD.
- Full-scale thermal hydrolysis & AD selected *ermB*, *ermF*, *mefA/E*, *qnrS* and *tetM*.
- Archaea biomass and community presented important impacts on the fate of ARGs.
- Total ARGs in the sludge are mainly affected by MGEs during the advanced AD.