Environment International 138 (2020) 105535

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Abundance and diversity of microbial arsenic biotransformation genes in the sludge of full-scale anaerobic digesters from a municipal wastewater treatment plant

Weiwei Zhai[a], Tianyue Qin[a], Liguan Li[b], Ting Guo[a], Xiaole Yin[b], Muhammad Imran Khan[c], Muhammad Zaffar Hashmi[d], Xingmei Liu[a], Xianjin Tang[a,*], Jianming Xu[a]

[a] Institute of Soil and Water Resources and Environmental Science, Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China
[b] Department of Civil Engineering, The University of Hong Kong, Hong Kong Special Administrative Region
[c] Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan
[d] Department of Chemistry, COMSATS University Islamabad, Pakistan

ARTICLE INFO

Handling Editor: Prof. Yong-Guan Zhu

Keywords:
Sludge
Anaerobic digestion
Metagenomic
qPCR
Arsenic biotransformation genes

ABSTRACT

Arsenic (As) is a potential contaminant in sewage sludge that may affect waste treatment and limit the use of these waste materials as soil amendments. Anaerobic digestion (AD) is an important and effective process for the treatment of sewage sludge and the chemical speciation of As is particularly important in sludge AD. However, the biotransformation genes of As in sludge during AD has not been fully explored. In this study, the influent and effluent sludge of anaerobic digester in a wastewater treatment plant (WWTP) was collected to investigate the species transformations of As, the abundance and diversity of As biotransformation genes was explored by real-time PCR (qPCR) and metagenomic sequencing, separately. The results showed that arsenite [As(III)] and arsenate [As(V)] were predominant in the influent sludge, whereas the relative abundance of monomethylarsenic acid (MMA) increased by 25.7% after digestion. As biotransformation genes were highly abundant, and the As (III) S-adenosylmethionine methyltransferase (*arsM*) gene was the predominant which significantly increased after AD by qPCR analysis. Metagenomic analysis indicated that the diversity of the *arsM*-like sequences also increased significantly after AD. Most of the *arsM*-like sequences in all the influent and effluent sludge samples were related to Bacteroidetes and Alphaproteobacteria. Furthermore, co-occurrence network analysis indicated a strong correlation between the microbial communities and As. This study provides a direct and reliable reference on As biotransformation genes and microbial community in the AD of sludge.

1. Introduction

Rapid industrialization and urbanization have resulted in the production of large amounts of sludge from municipal and industrial wastewater treatment plants (WWTP). Sludge is a heterogeneous material and usually has high water content (Huang et al., 2018). It also contains various contaminants, such as toxic metals, pathogens, and some organic compounds (Fonts et al., 2012; Westerhoff et al., 2015). Meanwhile, the large amounts of carbon and nutrients in sludge are worth recycling (Withers et al., 2015). Anaerobic digestion (AD) is one of the most effective treatment methods for sewage sludge. AD can transform the organic matter in sludge into biogas, reduce the amount of final sludge solids for disposal, destroy most of the pathogens present in sludge, and limit odor problems associated with residual matter (Gonzalez-Gil et al., 2016; Yuan and Zhu, 2016). However, without treatment, the high toxic metals contents in sludge may threaten the environment and become a key factor that limits the agricultural application of sludge because these elements are nondegradable and toxic (Dong et al., 2013; Wu et al., 2018). Among the metals, arsenic (As) is a typical element with high toxicity (Han et al., 2016) and may affect the sludge treatment process and limit the sludge application to soil. Reportedly, in batch AD, 56.7% of As is accumulated in anaerobic granular sludge (Zhang et al., 2014). Furthermore, high levels of As are known to be toxic to microorganisms in AD (Tang et al., 2017). The toxicity of As is known to depend on As species (Guillodmagnin et al., 2018), and As can be transformed from stable to bioavailable fractions in AD (Zhai et al., 2017b). Therefore, the chemical speciation of As is particularly important in wastewater treatment. However, to date, only

* Corresponding author.
E-mail address: xianjin@zju.edu.cn (X. Tang).

https://doi.org/10.1016/j.envint.2020.105535
Received 11 September 2019; Received in revised form 24 January 2020; Accepted 26 January 2020
Available online 26 March 2020

few studies have focused on the concentration and transformation of As species during the AD process of sludge in WWTPs.

The As oxidation, As(V) respiratory and cytoplasmic reduction, as well as As(III) methylation mediated by microorganism play an important role in the global As cycle in the environment (Lett et al., 2012). Genes involved in As biotransformation mainly consist of *aioA* genes [As(III) oxidation] (Lett et al., 2012), *arrA* genes [As(V) respiration reduction] (Saltikov et al., 2005), *arsC* genes [As(V) detoxification reduction] (Oremland and Stolz, 2005), and *arsM* genes [As(III) methylation] (Qin et al., 2006). As(III) oxidation gene *aioA* has been well characterized phylogenetically and ecologically widespread (Takafumi et al., 2018; Simona et al., 2017). Most As(III) oxidizers are mesophilic aerobes, and the final electron acceptor of the metabolic pathway is O_2. Respiratory arsenate reductase is a periplasmic dimethyl sulfoxide reductase having a catalytic subunit arrA and smaller subunit arrB, which catalysing As(V) respiration under anoxicconditions (Malasarn et al., 2008; Richey et al., 2009). The As resistance system is organized in the *ars* operon, which involves an arsenate reductase (*arsC*), an arsenite efflux pump (*arsB* or *ACR3*), and a transcriptional repressor (*arsR*) (Govarthanan et al., 2015). The arsenate reductase encoded by *arsC* gene is primarily used for carrying out the reduction of As(V) (Kumari and Jagadevan, 2016). Moreover, ArsM catalyzes the methylation of As (Zhao et al., 2013; Huang et al., 2018), and its gene can be used as a molecular marker for investigating As-methylating microbes (Jia et al., 2013).

Traditional molecular fingerprint methods, such as T-RFLP, DGGE, and TGGE, have been used to investigate the distribution and diversity of one or several subtypes of As metabolism genes (Jia et al., 2014, 2013; Schuchmann and Muller, 2014; Silver and Phung, 2005). Recently, with the development of high-throughput sequencing techniques, metagenomic is regarded as the most efficient, reliable, and accurate method to reveal the entire microbial composition and microbial metabolism synchronously under complex environment conditions. It has been successfully applied to provide reliable information on the community composition and identify the functional genes related to the metabolism of As (Thomas et al., 2012) in water, sediment, soil, and activated sludge (Bertin et al., 2011; Plewniak et al., 2013; Luo et al., 2014; Cai et al., 2013). For example, Costa et al. (2014) successfully identified a large number of phylogenetically distinct As-resistant bacterial genera from gold mining sediment using metagenomic analysis. Different from the sediments, the sludge after anaerobic digestion could be applied in agricultural soil as an excellent conditioner, which could improve the soil physical conditions, boost the soil microbial activity and enhance crop yields (Amoah et al., 2018; Zhang and Li, 2019). However, few studies have been conducted on assessing the composition of As metabolism genes and the diversity of microbes in the digested sludge using metagenomic method. Therefore, to reduce the possible exposure risk of As associated with agricultural application of digested sludge, a deep insight into the transformation of As species, the diversity of microbes and their functional genes during the AD process of sludge should be obtained.

In the present study, influent and effluent sludge during the AD treatment in a WWTP was collected to investigate the fate of As during treatment. The abundance and diversity of As biotransformation genes in the sludge samples were further explored. The findings of the present study will provide better understanding and important implications for the microbial-mediated As transformation during sludge AD process.

2. Materials and methods

2.1. Sludge sampling

The sludge was collected from a WWTP in Qingdao City, Shandong Province, China. Three influent and three effluent sludge samples were collected from an anaerobic digester of the WWTP. In order to avoid the accidental results, we collected samples in three different months (May, July, and September in 2016) as three parallel samples to ensure the results were true and credible. The samples were immediately transported to laboratory with ice bag. One part of the samples was then lyophilized, ground and sieved (< 60 mesh), and stored at −20 °C before further processing and analysis. Another part of samples was stored at −80 °C for DNA extraction.

2.2. Physiochemical properties of sludge

The physiochemical properties of sludge were determined following the published methods (Xiao et al., 2014a, 2014b). Chemical oxygen demand (COD) was measured by spectrophotometric method using a DR890 spectrophotometer based on HACH manual (HACH Company, USA). Moisture contents of the samples were measured after drying at 105 °C for 24 h. Total solids (TS) and volatile solids (VS) were measured according to the standard methods (APHA, 1998). The As and other toxic metals in the sludge samples were digested by a concentrated acid mixture containing 4 mL HNO_3, 2 mL HF and 2 mL of H_2O_2 using a microwave digester (MARS6, CEM Microwave Technology Ltd., USA). The digestion procedure was 0–20 min, 0–210 °C; 20–70 min, 210 °C. After digestion, the samples were filtered and diluted with deionized water, then determined through ICP-MS (ICP-MS NEXION300XX, PerkinElmer, Inc., USA). A soil reference (GBW 07429) purchased from the National Research Centre of China was used to determine the accuracy of the analytical method. The sample recoveries of toxic metals were within the range of 95.4–105.2% (n = 3). As speciation of dried sludge was extracted by 10 mL 2% H_3PO_4 and analyzed by high-performance liquid chromatography (HPLC, Series 200, PerkinElmer, Inc., USA) coupled with inductively coupled plasma mass spectrometry (ICP-MS) in accordance with the previously described method (Zhai et al., 2017a). However, since the amount of influent and effluent of wastewater could not be measured exactly, and the volatilized As in gas from the digester was also difficult to collect and analyze, no mass balance was conducted and the As species were shown in relative abundance in the present study.

2.3. DNA extraction and Illumina HiSeq sequencing

Sludge samples (50 mL) from each influent and effluent of the AD reactor were centrifuged at 3500*g* for 20 min (4 °C). Supernatant was removed, and pellets (0.25 g fresh weight) were used to extract the total DNA using a PowerSoil DNA Isolation Kit (MO BIO, USA) following the manufacturer's protocol. DNA concentration was estimated using a spectrophotometer (NanoDrop ND-2000, USA). DNA samples of the sludge were sent to Novogene Company (Beijing, China) for sequencing by Illumina HiSeq 2500. Two paired-end libraries (350 bp insert size) were constructed. Raw reads, with number of low-quality bases (Q < 38) above 40 and/or number of Ns (base call with low confidence) above 10 were removed (Chen et al., 2013). After removing reads of human contamination, total clean reads of 32,618.84 Mbp were generated with 5,457.73 Mbp reads per sample.

2.4. Bioinformatics analysis

To retrieve community composition from metagenomic datasets, clean reads were first assembled by SOAPdenovo using default parameters. The assembled contigs with length > 500 bp were retained for downstream analysis. ORFs were predicted by MetaGeneMark (Tang and Borodovsky, 2010) and searched against the National Center for Biotechnology Information (NCBI) nonredundant database using DIAMOND (e-value < 1e-10) (Buchfink et al., 2015). Taxonomy information of annotated genes for each ORF was obtained, and those ORFs with multiple annotations were cleaned by LCA algorithm to get the common taxonomy. Abundance of taxonomies were calculated by multiplying mapped reads to ORFs with read length (Karlsson et al., 2012, 2013; Li et al., 2014).

To analyze the distribution, diversity, and abundance of As functional gene groups, local BLASTX programs were used to align the trimmed clean reads of each dataset against an As metabolism protein database constructed previously (Cai et al., 2013), which includes 17 subdatabases corresponding to 17 subtypes of As biotransformation genes. The e-value cutoffs of 1e-5 were used to perform the BLAST search, and the following analysis was based on the strict criteria of aligned length ≥37 aa and aligned identity ≥90% to screen the BLASTX outputs against the As metabolism protein database. To minimize possible bias, we normalized the read number of recovered genetic candidates of As biotransformation by reference sequence length and 16S rRNA gene abundance. Abundance of potential As biotransformation genes was finally quantified as the ratio of the copy numbers of the As biotransformation genes to that of 16S rRNA genes. We annotated the taxonomies of the As biotransformation genes by retrieving the phylogenetic information from the source strains in NCBI database.

2.5. Real-time qPCR analysis

Real-time PCR (qPCR) was used to determine the quantitative distribution of As biotransformation genes (i.e., *aioA*, *arsC*, and *arsM*). Bacterial 16S rRNA gene was targeted to quantify the total bacterial population and normalize the abundance of As-related genes in the sludge samples. Primer pairs and PCR thermal programs conditions were conducted according to the previous study (Zhang et al., 2015b).

2.6. Statistical analysis

All experiments were conducted in triplicate, and physicochemical characteristics were expressed as mean ± standard deviation. One-way analysis of variance was performed to analyze the significant difference of characteristics between influent and effluent sludge during AD using SPSS 18.0, with significance defined at the 0.05 level, followed by Duncan's test and correlation analysis. Heatmap and clustering analyses were generated in R (3.1.2.) using ggplots package. Linear discriminant effect size (LEfSe) analysis cladogram was performed on http://huttenhower.sph.harvard.edu/lefse/ (Segata et al., 2011) on the basis of the absolute abundance of assigned taxa, and LDA > 3.6 was selected to indicate significant difference. A connection with a strong ($|r| > 0.6$) and significant ($P < 0.05$) Spearman's correlation was used to visualize the correlation between the abundant taxa (top 70 most abundant OTUs) and the contaminant fractions in the co-occurrence network.

3. Results and discussion

3.1. Evaluation of AD

Table S1 summarizes the pH, moisture content, COD, NH_4^+-N, VS, TS, and other characteristics of the influent and effluent sludge of AD during July, August and September 2016. The pH values were 6.2–7.4, within the permissible range for AD (Dong et al., 2013). The pH is an important parameter that affects AD as both methanogenic and acidogenic microorganisms have their optimal pH (Chen e al., 2008). The favorable pH for bacteria growth and to achieve maximal methane yield during AD was in the range of 6.8–7.2 (Suanon et al., 2017). The effluent samples showed more alkaline pH than the influent samples in all three months. The high pH may be due to the conversion of volatile fatty acids to CH_4 and CO_2 by methanogens and the degradation of nitrogenous compounds during AD (Yu et al., 2002). Biodegradable organic substances can be removed through conversion to methane and CO_2 during AD, thereby resulting in COD decrease in the digester. The COD removal efficiency after AD was in the range of 83%-85% during July, August and September 2016. The NH_4^+-N values increased significantly after AD in both influent and effluent during all the three months, which suggests the mineralization of proteins (Göblös et al., 2008). The VS/TS values decreased in the effluent samples, which could be explained by the microbial decomposition of organic matter and the stabilization during AD. In sum, the physicochemical characteristics of the sludge samples suggested the successful operation of AD in the WWTP.

3.2. Variation of total HMs during AD

The metals concentrations in the sludge samples followed the order of As < Ni < Pb < Cr < Cu < Zn (Table S1). The average concentrations of Cr, Cu, Ni, Pb, and Zn in the effluent sludge increased by 15.4%, 23.9%, 23.2%, 31.1%, and 29.5%, respectively, compared with that in the influent sludge. After AD, the As concentrations in the sludge also increased by 50.5%, 41.7%, and 7.0% in May, July, and September, respectively (Table S1), compared with that in the sludge before digestion. These results are consistent with those of Dong et al. (2013), who also reported an increase of metals concentrations in sludge after digestion. The increase of metals concentration could be explained by the weight loss during the AD which was caused by organic matter decomposition, fluids evaporation, aerosols capture and biogas release (Appels et al., 2008). Further, the accumulation of metals can also be the function of adsorption by the sludge (Li et al., 2016).

3.3. As species analysis

Speciation analysis was conducted by focusing on four As species: As(III), demethylarsinic acid (DMA), MMA, and As(V). The relative distributions of the four species during AD are shown in Fig. 1 (for their concentrations data, see Supporting Information, Fig. S1). Inorganic As [As(V) and As(III)] were the main species in the influent samples, accounting for 41.7–74.3% and 13.7–45.8%, respectively. As(V) has been reported as the most abundant As in fresh sludge due to coagulation treatment (Meng et al., 2001). Under anaerobic condition, As(V) was reduced to As(III) and thus significantly decreased to 9.8%, 7.1%, and 5.2% in the effluent samples in May, July, and September, respectively. Interestingly, the As(III) concentrations also decreased by 8.9%, 17.9%, and 19.4% during these months. This decrease in As(III) could be explained by the formation of MMA by methylation process (Li et al., 2015). As(V) was the main species in the effluent samples (36.5–64.4%), followed by MMA (28.7–34.6%). In the influent samples, MMA constituted a small proportion of the total concentrations of As (8.0%, 6.8%, and 5.9%). However, after AD, a significant increase in MMA (i.e., 28.7%, 34.6%, and 34.6%) was observed in the effluent samples. The DMA was minimal of the four species in the sludge. In the influent of AD, the DMA relative abundance was 4.0%, 4.4%, and 6.5% in May, July, and September, respectively. The different distribution of

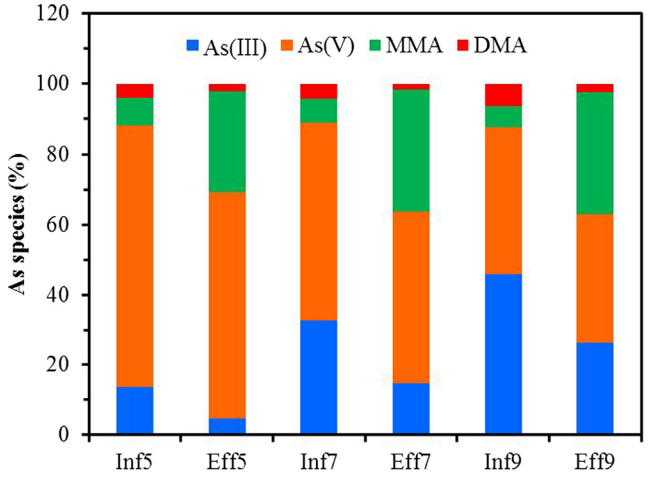

Fig. 1. Percent contribution of As species in influent and effluent sludge of AD.

As species suggested that this may be due to different conditions and microbes in the influent samples of AD. Previous studies indicated that MMA and DMA showed dynamic trends under anerobic conditions. As expected from the results obtained with the mixtures of As species under aerobic condition, a part of MMA was biologically methylated to DMA. However, a part of DMA was also demethylated to MMA. This indicated the simultaneous occurrence of methylation and demethylation of DMA. According to Sierra-Alvarez et al. (2006), the MMA was main biotransformation product identified from DMA metabolism, and the molar yields ranging from 8% to 65%. The removal of DMA was about 85% after 70 days of incubation in wet soil (Gao and Burau, 1997). It is suggested that demethylation are important steps in the anaerobic bioconversion of DMA and explained the high contents of MMA in the samples. After the AD process, the DMA decreased slightly to 2.1%, 1.7%, and 2.5%. It caused by that the generated DMA was converted to TMA, which was released into the atmosphere with the methane (Webster et al., 2016). Mohapatra et al. (2008) reported that up to 35% of inorganic As volatilized in the effect of methanogenic bacteria in anaerobic sewage sludge digester. In addition, previous studies have found that pH and redox potential (Eh) have a strong effect on the solubility and bioavailability of As by control As speciation (Quazi et al., 2010). At neutral and alkaline pH values, the occurrence of As methylation in sludge suspensions is evident, and the loss of volatile arsines can be a significant As removal pathway (Carbonell-Barrachina et al., 2000). Previous study showed that the main part of As in the raw sludge was in the liquid part, while, the main part of As in the anaerobically digested samples was in the solid part of the sludge, low amounts of As were volatilized, and < 4% of the As was in the aqueous phase (Webster et al., 2016). As a result, the application of anaerobically digested sludge could be likely to increase the bioaccumulation rate of As in the crops and induce more human health risk (Reza et al., 2018). However, the volatilized As was not collected in the present study, the mass balance of As should be further studied in the future. Moreover, since the As(III) methylation is a detoxification pathway, more technologies should be developed to enhance the As volatilization and methylation during the sludge anaerobic digestion and thereby reduce the exposure risk of As associated with the application of anaerobically digested sludge.

3.4. Abundance of As biotransformation genes

As transformations is considerably influenced by microorganisms. The abundance of functional genes related to As biotransformation was quantified using qPCR. The copy numbers of the *aioA*, *arsC*, and *arsM* genes (Fig. 2) were normalized to 16S rRNA genes to minimize the variances caused by different background bacterial abundance, extractions, and analytical efficiencies. The highest relative abundance of *aioA* gene was found in the influent samples (i.e., 3.1–9.0 × 10^{-5}, Fig. 2A), and it significantly decreased by 47.4%, 73.6%, and 60.4% in the effluent samples, As(III) oxidation has been reported as the microbial detoxification metabolism in various environments because As(V) is less toxic than As(III) (Yan et al., 2018). Previous study reported that AioA catalyzed the aerobic As(III) oxidation, and insufficient oxygen in AD decreased *aioA* gene abundance (Duval et al., 2008). While it has been reported that anoxic chemolithoautotrophic strains were able to oxidize As(III) via complete denitrification of nitrate to dinitrogen gas (Zhang et al., 2017a). *ArsC* gene can reduce As(V) to As(III) and is more widespread among anaerobic and aerobic microbes (Silver and Phung, 2005). However, it had the lowest abundance, with 0.15–1.98 × 10^{-6} and was mainly detected in the influent samples (Fig. 2B), which indicated that the microorganisms that reduced As(V) under aerobic conditions may be more abundant than the anaerobic microorganisms. It is well known that As(III) methylation mediated by ArsM is a detoxification mechanism in microorganisms (Zhang et al., 2015a). As shown in Fig. 2C, the *arsM* gene with 1.1–2.1 × 10^{-4} had the highest mean relative abundance in the influent sample of AD, whereas the

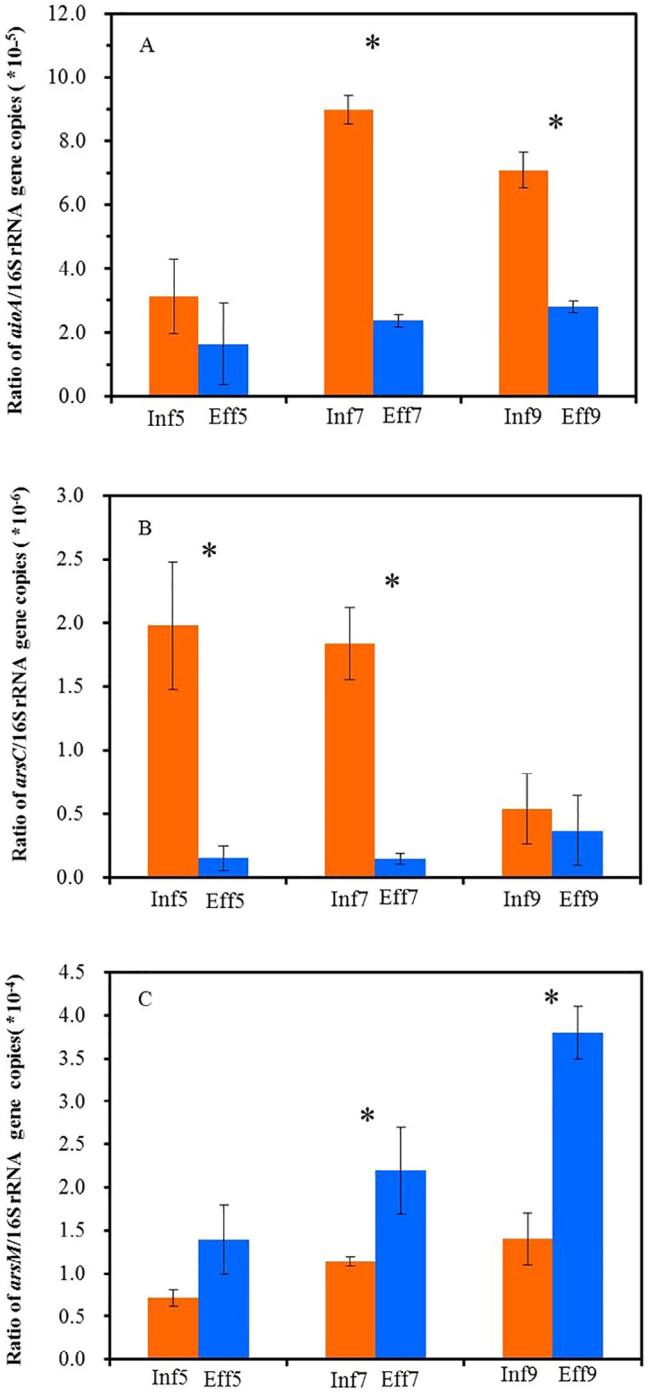

Fig. 2. Relative abundances of *aioA* (A), *arsC* (B), and *arsM* (D) in influent and effluent sludge during AD. *AioA*, *arsC*, and *arsM* were normalized to that of 16S rRNA genes. The asterisk indicates significant differences between influent and effluent sludge ($P < 0.05$).

relative abundance of the *arsM* genes in the effluent samples of the anaerobic digester showed a significant increase (i.e., 3.4–6.3 × 10^{-4}), implying a strong potential to produce methylated As species. *ArsM* gene copies have been investigated mostly in soil in previous studies. For example, it has been reported that *arsM* genes constituted 6–9% of the total As biotransformation genes among five paddy soils and can even be found in an As-free sample of activated sludge (Xiao et al., 2016; Cai et al., 2013; Luo et al., 2014). Jia et al. (2013) found that the copy numbers of *arsM* in paddy soil were in the order of 10^7–10^8 copies g^{-1} dry soil. The abundance of *arsM* is the highest among other As

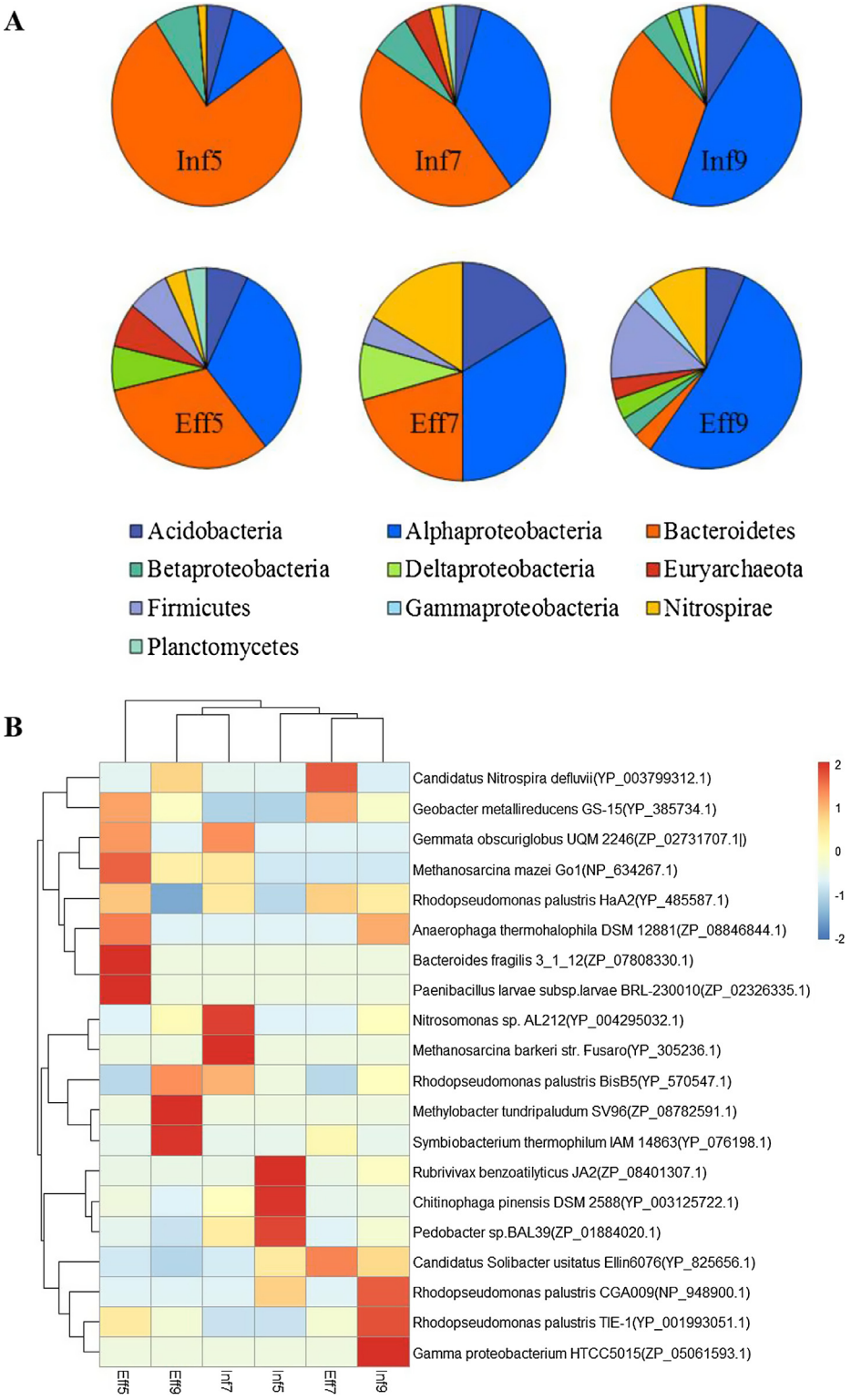

Fig. 3. Diversity of microbe involved in As(III) methylation in influent and effluent sludge. Taxonomic composition of annotated As methylation-related genes (A). Heatmap of *arsM* gene in influent and effluent sludge, and phylogenetic analysis using full-length protein sequences derived from *arsM* sub-database (phylogenetic tree on the left, and strain source with GenBank ID on the right (B).

biotransformation genes in the samples of swine wastewater treated by AD (Zhai et al., 2017b). A previous study reported that the *arsM* copy numbers correlated positively with soil pH (Zhao et al., 2013). In the present study, the increase of pH after AD stimulated the As methylation and improved the abundance of *arsM* genes. Anaerobic or low oxygen conditions favor the conversion of inorganic As into MMA and DMA by microorganisms (Ascar et al., 2008). The results suggested that the low oxygen condition in the AD reactor could affect the *arsM* abundance positively.

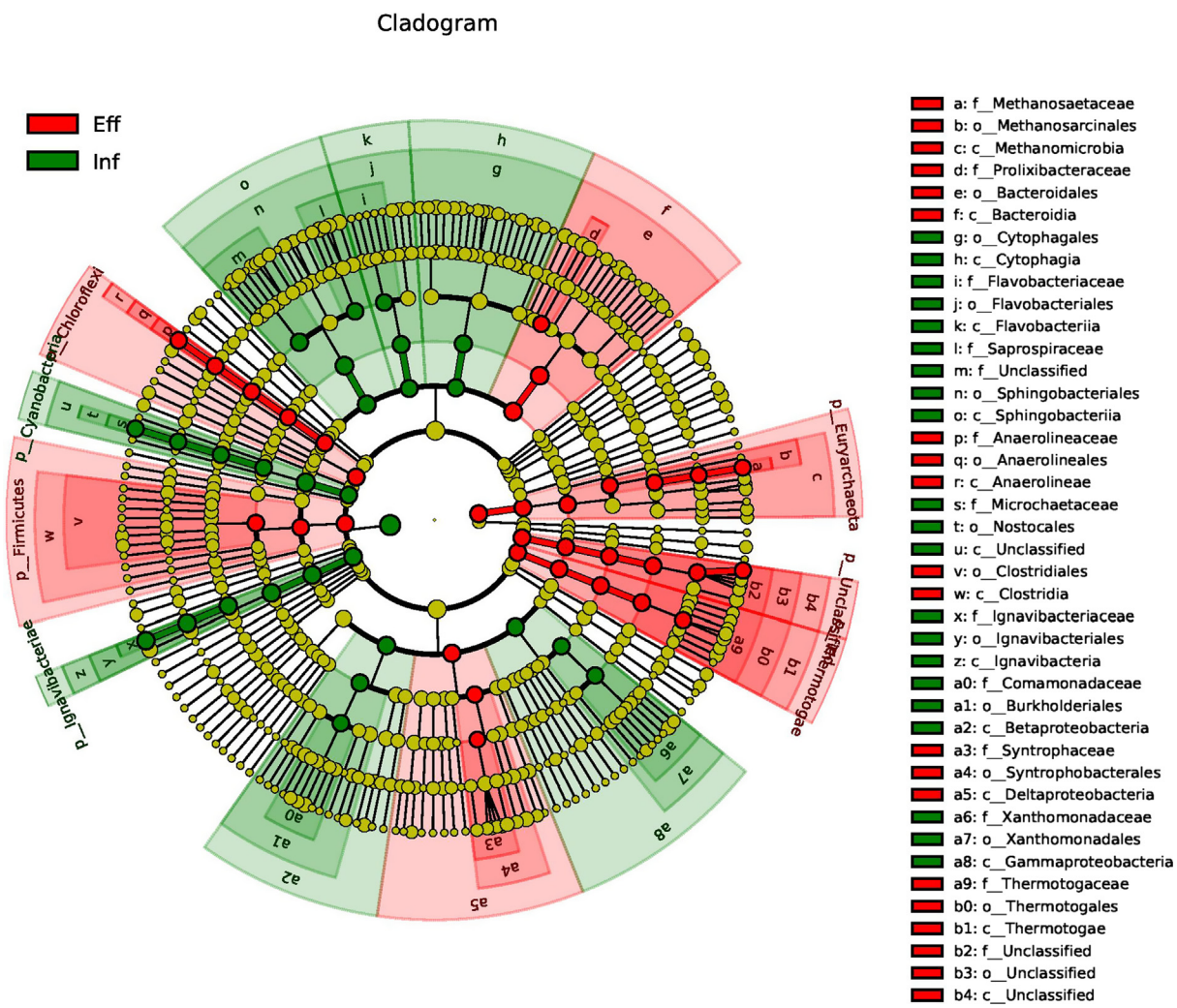

Fig. 4. LEfSe analysis cladogram of comparison results between influent and effluent sludge of anaerobic digester. Green and red circles represent the taxa that were abundant in the influent and effluent sludge, respectively; only the taxa that meet the LDA significance threshold of 3.6 are shown. The seven rings of the cladogram stand for domain (innermost), phylum, class, order, family, genus, and species. Inf: Influent sludge of anaerobic digester; Eff: Effluent sludge of anaerobic digester. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Diversity of arsM-like sequences via metagenomic analysis

Since As methylation is one of the main detoxification pathways for As in the environment and its gene *arsM* has been identified in a broad spectrum of phylogenies (Qin et al., 2006; Zhao et al., 2013), we applied metagenomic analysis to give a further insight into *arsM* diversity. The results showed that *arsM* sequences are quite different in three samples collected in May, July, and September, which may due to the variation of the influent in different months. Among the detected *arsM* sequences, most of the *arsM* sequences in the influent and effluent sludge samples belonged to Bacteroidetes and Alphaproteobacteria (Fig. 3A). Only few *arsM* sequences were related to Betaproteobacteria and Acidobacteria in the influent sludge. Meanwhile, after AD, the *arsM* sequences from Bacteroidetes decreased while *arsM* sequences from Alphaproteobacteria, Deltaproteobacteria, Firmiccutes, and Nitrospirae increased, suggesting that the genes encoding ArsM were widespread during AD. These results were consistent with the previous studies, which reported that Alphaproteobacteria and Deltaproteobacteria were the most abundant microbe that carrying *arsM* gene in estuarine and marine sediments (Zhang et al., 2017b; Guo et al., 2019). The most abundant *arsM* sequences in the influent and effluent sludge belonged to *Pedobacter* sp. BAL39 (ZP_01884020.1) and *Rhodopseudomonas palustris*, respectively (Fig. 3B). *R. palustris* was reported to have high methylation rate (Chen et al., 2014). The relative abundance of the *arsM* (> 50%) in the sludge was higher than those in soil, activated sludge, and coastal sediments (Cai et al., 2013). This result could be mainly attributed to the unique biogeochemical conditions in AD, such as the abundance of sulfate-reducing bacteria and methanoarchaea, which favors As methylation (Webster et al., 2016). Since both qPCR and metagenome were DNA based technologies, the profile of As biotransformation potential observed here might not imply microbial activities in real environment. The results presented here can be further complemented by metatranscroptomic and metaproteomic studies in future, which however is out of scope of this study.

3.6. Changes of microbial communities based on metagenomic datasets

In order to analyze the influence of anaerobic digestion on microbial community structure, we adopted linear discriminant analysis (LDA) effect size (LEfSe) from the phylum to species level separately to reveal the remarkable differences among the influent and effluent sludge of AD (Fig. 4). The results were shown that the bacterial communities in the influent sludge were significantly different from those in the effluent sludge. The influent samples from three different months shared similar phyla-level profiles where Bacteroidetes (29.3–38.6%), Proteobacteria (16.3–24.1%), and Ignavibacteriae (6.2–16.9%) were the

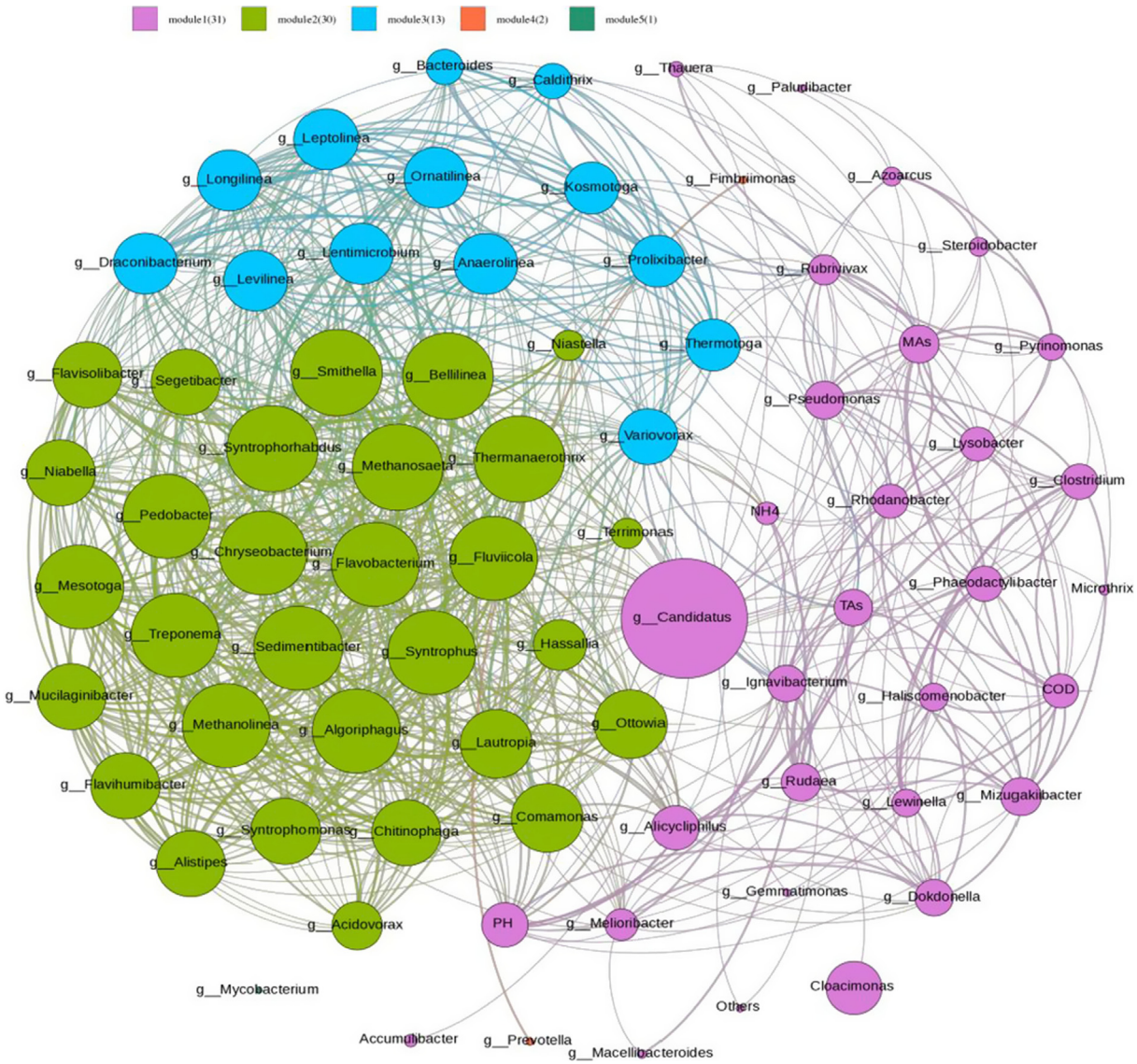

Fig. 5. Co-occurrence networks showing the correlation between bacterial taxa and environmental factors as nodes colored by modularity class. Edges are only shown for strong ($|r| > 0.6$) and significant ($P < 0.05$) Spearman's correlations.

predominant phyla. However, the relative abundance of Bacteroidetes decreased to 8.9%, 6.5%, and 11.3% after AD in May, July, and September, respectively (Supporting Information, Fig. S2). Bacteroidetes are frequently detected in anaerobic reactors and known to play important roles as fermenters and acidogens (Jang et al., 2014). Similarly, Proteobacteria decreased significantly after AD. They widely exist in the environment and can tolerate extremely severe environments, and most Alpha-, Beta-, Gamma-, and Delta-proteobacteria are known to be involved in the glucose, propionate, butyrate, and acetate utilization (Ariesyady et al., 2007). The relative abundance of Chloroflexi, Firmicutes, and Thermotogae populations increased significantly and became dominant after AD.

Furthermore, LEfSe analysis showed that, at the family level, 11 families exhibited significant changes (Fig. 4, LDA > 3.6) after AD. Flavobacteriaceae, Saprospiraceae, Microchaetaceae, Ignavibacteriaceae, Comamonadaceae, and Xanthomonadaceae decreased significantly after AD. Methanosaetaceae, Prolixibacteraceae, Anaerolineaceae, Syntrophaceae, and Thermotogaceae increased significantly in the effluent sludge compared with that in the influent. Recently, the phylogenetic diversity of municipal digesters for treating waste sludge has been reported using metagenomics sequencing (Yang et al., 2014; Guo et al., 2015; Cai et al., 2016). However, the characteristics of influent sludge and the operational conditions of AD have been reported to strongly influence the microbial community structure. Yang et al. (2014); Guo et al. (2015) showed that Proteobacteria was the most dominant phylum in anaerobic reactor digesting activated sludge from wastewater treatment, followed by Firmicutes, Bacteroidetes, and Actinobacteria. Meanwhile, Sundberg et al. (2013) found that Firmicutes were the most abundant in the co-digesting reactor containing various combinations of wastes from restaurants, households, and slaughterhouses. The dominant populations belong to the phyla Chloroflexi and Proteobacteria in the anaerobic digesters fed with various feed stocks. In this work, a fraction of industrial wastewater was fed into the activated sludge process, subsequently influencing the microbial community structure of the sludge in the anaerobic digester.

3.7. Correlation between environmental factors and microbial communities

Further investigations on the effects of environmental factors on microbial communities and biotic interactions were conducted by co-occurrence network analysis (Fig. 5). This network consists of 80 nodes, including 70 most abundant genera and 5 environmental factors. Three

major modules were considered in the modular network. Interestingly, all environmental factors and 26 genera co-occurred in Module 1. Meanwhile, 30 and 13 genera were clustered in Modules 2 and 3. Previous studies have reported that in the co-occurrence network, modules containing highly interconnected nodes may share similar functions, and the potential functions of the microbial community can be predicted on the basis of the functions of the located module (Ma et al., 2018). The hub in each module might be used as an indicator to predict the potential behavior of other nodes in the same module. Among the identified genera in Module 1, many members, including Ignavibacterium, Steroidobacter, Clostridium, Rhodanobacter, and Pseudomonas were correlated with total As and methylated As and environmental factors such as NH_4^+-N, COD and pH. Several genera, including Bacteroides, Clostridium, and Caldithrix were positively correlated with methylated As, whereas Pseudomonas, Steroidobacter, Phaeodactylibacter, Rhodanobacter, and Rubrivivax were negatively correlated with methylated As. Members of Clostridium and Pseudomonas have been identified as As(III)-methylation bacteria (Zhang et al., 2015a; Qiao et al., 2017). These observations suggest that the genera in Module 1 may be associated with As methylation and NH_4^+-N, COD, and pH. Furthermore, the microbial communities in AD related to As methylation were abundant.

4. Conclusions

The present study investigated the fate of As, the abundance and diversity of As biotransformation genes, as well as microbial communities during AD of the sludge. The results indicated that the fate of As highly influenced after AD. The As(III) and As(V) were the predominant forms in the influent sludge, whereas MMA significantly increased after AD, suggesting the methylation of As during the AD of sludge. The qPCR and metagenomic analysis results showed that As biotransformation genes were highly abundant, and the *arsM* gene was the most predominant with high diversity. Co-occurrence network analysis indicated a strong correlation between the microbial communities and As (MAs and TAs). Several taxa were identified as core genera due to their positive correlation with contaminant As species. This study provides an overall picture of As biotransformation genes in the AD of sludge, which is essential in understanding the fate and assessing the risk of As in sludge when applied to soil.

CRediT authorship contribution statement

Weiwei Zhai: Methodology, Writing - original draft, Writing - review & editing. **Tianyue Qin:** Methodology, Writing - original draft. **Liguan Li:** Software, Data curation, Writing - review & editing. **Ting Guo:** Data curation, Writing - review & editing. **Xiaole Yin:** Software, Data curation. **Muhammad Imran Khan:** Writing - review & editing. **Muhammad Zaffar Hashmi:** Writing - review & editing. **Xingmei Liu:** Validation, Writing - review & editing. **Xianjin Tang:** Conceptualization, Project administration, Funding acquisition, Supervision, Resources. **Jianming Xu:** Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (41671312).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105535.

References

Amoah, I.D., Adegoke, A.A., Stenstrom, T.A., 2018. Soil-transmitted helminth infections associated with wastewater and sludge reuse: a review of current evidence. Trop. Med. Int. Health. 23 (7), 692–703.
APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington DC.
Appels, L., Baeyens, J., Degrève, Jan, Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. Energ. Combus. 34 (6), 755–781.
Ariesyady, H.D., Ito, T., Okabe, S., 2007. Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester. Water Res. 41 (7).
Ascar, L., Ahumada, I., Richter, P., 2008. Influence of redox potential (Eh) on the availability of arsenic species in soils and soils amended with biosolid. Chemosphere 72 (10), 1548–1552.
Bertin, P.N., Heinrich-Salmeron, A., Pelletier, E., Goulhen-Chollet, F., Arsène-Ploetze, Florence, Gallien, Sébastien, et al., 2011. Metabolic diversity among main microorganisms inside an arsenic-rich ecosystem revealed by meta- and proteo-genomics. ISME. J. 5 (11), 1735–1747.
Buchfink, B., Xie, C., Huson, D.H., 2015. Fast and sensitive protein alignment using DIAMOND. Nat. Methods 12, 59–60.
Cai, L., Yu, K., Yang, Y., Chen, B.W., Li, X.D., Zhang, T., 2013. Metagenomic exploration reveals high levels of microbial arsenic metabolism genes in activated sludge and coastal sediments. Appl. Microbiol. Biot. 97 (21), 9579–9588.
Cai, M., Wilkins, D., Chen, J., Ng, S.K., Lu, H., Jia, Y., Lee, P.K., 2016. Metagenomic reconstruction of key anaerobic digestion pathways in municipal sludge and industrial wastewater biogas-producing systems. Front. Microbiol. 7, 778.
Carbonell-Barrachina, A.A., Jugsujinda, A., Burlo, F., Delaune, R.D., Patrick Jr., W.H., 2000. Arsenic chemistry in municipal sewage sludge as affected by redox potential and pH. Water Res. 34 (1), 216–224.
Chen, J., Sun, G.X., Wang, X.X., Vd, L., Rosen, B.P., Zhu, Y.G., 2014. Volatilization of arsenic from polluted soil by pseudomonas putida engineered for expression of the *arsM* As(III) S-adenosine methyltransferase gene. Environ. Sci. Technol. 48 (17), 10337–10344.
Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresource Technol. 99 (10), 4044–4064.
Costa, Patrícia S., Scholte, L.L.S., Reis, M.P., Chaves, A.V., Oliveira, P.L., Itabayana, L.B., et al., 2014. Bacteria and genes involved in arsenic speciation in sediment impacted by long-term gold mining. PLoS ONE 9 (4), e95655.
Dong, B., Liu, X., Dai, L., Dai, X., 2013. Changes of heavy metal speciation during high-solid anaerobic digestion of sewage sludge. Bioresource. Technol. 131, 152–158.
Duval, S., Ducluzeau, A.L., Nitschke, W., Schoepp-Cothenet, B., 2008. Enzyme phylogenies as markers for the oxidation state of the environment: the case of respiratory arsenate reductase and related enzymes. BMC Evol. Biol. 8 (1), 206–220.
Fonts, I., Gea, G., Azuara, M., Javier ábrego, Jesús Arauzo, 2012. Sewage sludge pyrolysis for liquid production: a review. Renew. Sust. Energ. Rev. 16 (5), 2781–2805.
Plewniak, Frédéric, Koechler, S., Navet, B., Dugat-Bony, E., Bertin, P.N., 2013. Metagenomic insights into microbial metabolism affecting arsenic dispersion in mediterranean marine sediments. Mol. Ecol. 22 (19), 4870–4883.
Gao, S., Burau, R.G., 1997. Environmental factors affecting rates of arsine evolution from and mineralization of arsenicals in soil. J. Environ. Qual. 26 (3), 753–763.
Göblös, S., Portörő, P., Bordás, D., Kálmán, M., Kiss, I., 2008. Comparison of the effectivities of two-phase and single-phase anaerobic sequencing batch reactors during dairy wastewater treatment. Renew. Energ. 33 (5), 960–965.
Gonzalez-Gil, L., Papa, M., Feretti, D., Ceretti, E., Mazzoleni, G., Steimberg, N., Pedrazzan, R., Bertanza, G., Lema, J.M., Carballa, M., 2016. Is anaerobic digestion effective for the removal of organic micropollutants and biological activities from sewage sludge? Water Res. 102, 211–220.
Govarthanan, M., Lee, S.M., Kamala-Kannan, S., Oh, B.T., 2015. Characterization, real-time quantification and in silico modeling of arsenate reductase (*arsC*) genes in arsenic-resistant *Herbaspirillum* sp. GW103. Res. Microbiol. 166 (3), 196–204.
Guillodmagnin, R., Brüschweiler, B.J., Aubert, R., Haldimann, M., 2018. Arsenic species in rice and rice-based products consumed by toddlers in switzerland. Food Addit. Contam. A. 1–15.
Guo, J., Peng, Y., Ni, B.J., Han, X., Lu, F., Yuan, Z., 2015. Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing. Microb. Cell. Fact. 14 (1), 33.
Guo, T., Li, L.G., Zhai, W.W., Xu, B.L., Yin, X.L., He, Y., Xu, J.M., Zhang, T., Tang, X.J., 2019. Distribution of arsenic and its biotransformation genes in sediments from the East China Sea. Environ. Pollut. 635, 995–1003.
Han, H., Hu, S., Lu, C., Wang, Y., Jiang, L., Xiang, J., Su, S., 2016. Inhibitory effects of Cao/Fe_2O_3 on arsenic emission during sewage sludge pyrolysis. Bioresource. Technol. 218, 134–139.
Huang, K., Xu, Y., Packianathan, C., Gao, F., Chen, C., Zhang, J., Shen, Q., Rosen, B.P., Zhao, F., 2018a. Arsenic methylation by a novel *arsM* As(III) S-adenosylmethionine methyltransferase that requires only two conserved cysteine residues. Mol. Microbiol. 107 (2), 265–276.
Huang, R., Zhang, B., Saad, E.M., Ingall, E.D., Tang, Y., 2018b. Speciation evolution of

zinc and copper during pyrolysis and hydrothermal carbonization treatments of sewage sludges. Water Res. 132, 260–269.
Jang, H.M., Kim, J.H., Ha, J.H., Park, J.M., 2014. Bacterial and methanogenic archaeal communities during the single-stage anaerobic digestion of high-strength food wastewater. Bioresource. Technol. 165, 174–182.
Jia, Y., Zhang, L.M., Huang, H., Zhong, M., Zhu, Y.G., 2013. Microbial arsenic methylation in soil and rice rhizosphere. Environ. Sci. Technol. 47 (7), 3141–3148.
Karlsson, F.H., Tremaroli, V., Nookaew, I., Bergstrom, G., Behre, C.J., Fagerberg, B., Nielsen, J., Backhed, F., 2013. Gut metagenome in European women with normal, impaired and diabetic glucose control. Nature 498 (7452), 99–103.
Karlsson, F.H., FaK, F., Nookaew, I., Tremaroli, V., Fagerberg, B., Petranovic, D., Backhed, F., Nielsen, J., 2012. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat. Commun. 3, 1245.
Kumari, N., Jagadevan, S., 2016. Genetic identification of arsenate reductase and arsenite oxidase in redox transformations carried out by arsenic metabolising prokaryotes-a comprehensive review. Chemosphere 163, 400–412.
Lett, M.C., Muller, D., Lièvremont, Didier, Silver, S., Santini, J., 2012. Unified nomenclature for genes involved in prokaryotic aerobic arsenite oxidation. J. Bacteriol. 194 (2), 207–208.
Li, J.B., Rui, J.P., Yao, M.J., Zhang, S.H., Yan, X.F., Wang, Y.P., Yan, Z.Y., Li, X.Z., 2015. Substrate type and free ammonia determine bacterial community structure in full-scale mesophilic anaerobic digesters treating cattle or swine manure. Front. Microbiol. 6, 1337.
Li, J., Jia, H., Cai, X., et al., 2014. An integrated catalog of reference genes in the human gut microbiome. Nat. biotechnol. 32 (8), 834–841.
Li, J., Yuan, S., Wang, W., Ji, F., Hu, Z., 2016. Adsorption characteristics of 4-hydroxy-3-aminophenylarsonic acid (HAPA) onto anaerobic granular sludge. Desalin. Water Treat. 57, 20714–20725.
Luo, J., Bai, Y., Liang, J., Qu, J., 2014. Metagenomic approach reveals variation of microbes with arsenic and antimony metabolism genes from highly contaminated soil. PLoS ONE 9 (10), e108185.
Ma, B., Zhao, K., Lv, X., Su, W., Dai, Z., Gilbert, J.A., Brookes, P.C., Faust, K., Xu, J., 2018. Genetic correlation network prediction of forest soil microbial functional organization. ISME. J. 12 (10), 2492–2505.
Malasarn, D., Keeffe, J.R., Newman, D.K., 2008. Characterization of the arsenate respiratory reductase from shewanella sp. strain ANA-3. J. Bacteriol. 190 (1), 135–142.
Meng, X., Korfiatis, G.P., Jing, C., Christodoulatos, C., 2001. Redox transformations of arsenic and iron in water treatment sludge during aging and TCLP extraction. Environ. Sci. Technol. 35 (17), 3476–3481.
Mohapatra, D., Mishra, D., Chaudhury, G.R., Das, R.P., 2008. Removal of arsenic from arsenic rich sludge by volatilization using anaerobic microorganisms treated with cow dung. J. Soil. Contam. 17 (3), 11.
Oremland, R.S., Stolz, J.F., 2005. Arsenic, microbes and contaminated aquifers. Trends. Microbiol. 13, 45–49.
Qin, J., Rosen, B.P., Zhang, Y., Wang, G., Franke, S., Rensing, C., 2006. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. P. Natl. Acad. Sci. USA 103, 2075–2080.
Qiao, J.T., Li, X.M., Li, F.B., 2017. Roles of different active metal-reducing bacteria in arsenic release from arsenic-contaminated paddy soil amended with biochar. J. Hazard. Mater. 344, 958–967.
Quazi, S., Sarkar, D., Datta, R., 2010. Effect of soil aging on arsenic fractionation and bioaccessibility in inorganic arsenical pesticide contaminated soils. Appl. Geochem. 25 (9), 1422–1430.
Reza, S.H., Mahmood, A., Samane, M., Kazem, N., Ramin, N., Changani, K.F., et al., 2018. Assessment of the health risk induced by accumulated heavy metals from anaerobic digestion of biological sludge of the lettuce. Biol. Trace. Ele. Res. 188, 514–520.
Richey, C., Chovanec, P., Hoeft, S.E., Oremland, R.S., Basu, P., Stolz, J.F., 2009. Respiratory arsenate reductase as a bidirectional enzyme. Biochem. Biophys. Res. Commun. 382 (2).
Saltikov, C.W., Wildman Jr., R.A., Newman, D.K., 2005. Expression dynamics of arsenic respiration and detoxification in Shewanella sp. strain ANA-3. J. Bacteriol. 187 (21), 7390–7396.
Schuchmann, K., Muller, V., 2014. Autotrophy at the thermodynamic limit of life: amodel for energy conservation in acetogenic bacteria. Nat. Rev. Microbiol. 12, 809–821.
Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome. Biol. 12, R60.
Sierra-Alvarez, R., Yenal, U., Field, J.A., Kopplin, M., Gandolfi, A.J., Garbarino, J.R., 2006. Anaerobic biotransformation of organoarsenical pesticides monomethylarsonic acid and dimethylarsinic acid. J. Agr. Food. Chem. 54 (11), 3959–3966.
Silver, S., Phung, L.T., 2005. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. Appl. Environ. Microb. 71 (2), 599–608.
Simona, C., Sarah, Z., Stefano, A., Stefano, F., Barbara, C., Anna, C., Lucia, C., Simona, R., 2017. Phylogenetic structure and metabolic properties of microbial communities in arsenic-rich waters of geothermal origin. Front. Microbiol. 8, 2468.
Suanon, Fidèle, Sun, Q., Li, M., Cai, X., Zhang, Y., Yan, Y., et al., 2017. Application of nanoscale zero valent iron and iron powder during sludge anaerobic digestion: impact on methane yield and pharmaceutical and personal care products degradation. J. Hazard. Mater. 321, 47–53.
Sundberg, C., Al-Soud, W.A., Larsson, M., Alm, E., Yekta, S.S., Svensson, B.H., et al., 2013. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. FEMS Microbiol. Ecol. 85 (3), 612–626.
Takafumi, K., Satoshi, M., Natsuko, H., 2018. Influence of the chemical form of antimony on soil microbial community structure and arsenite oxidation activity. Microbes. Environ. 33 (2), 214–221.
Tang, L., Feng, H., Tang, J., Zeng, G., Deng, Y., Wang, J., Liu, Y., Zhou, Y., 2017. Treatment of arsenic in acid wastewater and river sediment by Fe@Fe_2O_3, nanobunches: the effect of environmental conditions and reaction mechanism. Water Res. 117, 175–186.
Tang, S., Borodovsky, M., 2010. Ab Initio Gene Identification in Metagenomic Sequences. Nucleic Acids Res. 38, e132.
Thomas, T., Gilbert, J., Meyer, F., 2012. Metagenomics - a guide from sampling to data analysis. Microb. Inform. Exp. 2 (1), 3.
Webster, T.M., Reddy, R.R., Tan, J.Y., Nostrand, J.D.V., Raskin, L., 2016. Anaerobic disposal of arsenic-bearing wastes results in low microbially mediated arsenic volatilization. Environ. Sci. Technol. 50 (20), 10951–10959.
Westerhoff, P., Lee, S., Yang, Y., Gordon, G.W., Hristovski, K., Halden, R.U., Herckes, P., 2015. Characterization, recovery opportunities, and valuation of metals in municipal sludges from U.S. wastewater treatment plants nationwide. Environ. Sci. Technol. 49 (16), 9479.
Withers, P.J.A., Elser, J.J., Hilton, J., Ohtake, H., Schipper, W.J., Van Dijk, K.C., 2015. Cheminform abstract: greening the global phosphorus cycle: How green chemistry can help achieve planetary P sustainability. J. Cheminform. 46 (23), 2087–2099.
Wu, D., Yu, X., Chu, S., Jacobs, D.F., Wei, X., Wang, C., Long, F., Chen, X., Zeng, S., 2018a. Alleviation of heavy metal phytotoxicity in sewage sludge by vermicomposting with additive urban plant litter. Sci. Total Environ. 633, 71.
Wu, Y., Cui, E., Zuo, Y., Cheng, W., Chen, H., 2018b. Fate of antibiotic and metal resistance genes during two-phase anaerobic digestion of residue sludge revealed by metagenomic approach. Environ. Sci. Pollut. Res. 25 (14), 1–8.
Xiao, K., Li, L., Ma, L., Zhang, S., Bao, P., Zhang, T., Zhu, Y., 2016. Metagenomic analysis revealed highly diverse microbial arsenic metabolism genes in paddy soils with low-arsenic contents. Environ. Pollut. 211, 1–8.
Xiao, J.N., Zhang, L., Zheng, L.N., Wang, L., Wei, H.F., Zhang, M., 2014a. Study on the characteristics of biological phosphorus removal sludge in different operation mode of reactor. Adv. Mater. Res. 1073–1076, 860–865.
Xiao, Z., Zhu, X., Xi, L., Hou, X., Li, F., Lu, J.R., 2014b. Biodegradation of C5–C8 fatty acids and production of aroma volatiles by Myroides sp. ZB35 isolated from activated sludge. J. Microb. 52 (5), 407–412.
Yan, G., Chen, X., Du, S., Deng, Z., Wang, L., Chen, S., 2018. Genetic mechanisms of arsenic detoxification and metabolism in bacteria. Curr. Genet. 5, 1–10.
Yang, Y., Yu, K., Xia, Y., Lau, F.T.K., Zhang, T., 2014. Metagenomic analysis of sludge from full-scale anaerobic digesters operated in municipal wastewater treatment plants. App. Microb. Biotechnol. 98 (12), 5709–5718.
Yu, H.Q., Fang, H.H.P., Gu, G.W., 2002. Comparative performance of mesophilic and thermophilic acidogenic upflow reactors. Process Biochem. 38 (3), 447–454.
Yuan, H., Zhu, N., 2016. Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. Renew. Sust. Energ. Rev. 58, 429–438.
Zhai, W., Wong, M.T., Luo, F., Hashmi, M.Z., Liu, X., Edwards, E.A., Tang, X., Xu, J., 2017a. Arsenic methylation and its relationship to abundance and diversity of arsm genes in composting manure. Sci. Rep-UK 7, 42198.
Zhai, W., Qin, T., Guo, T., Khan, M.I., Tang, X., Xu, J., 2017b. Arsenic Transformation in Swine Wastewater with Low-Arsenic Content during Anaerobic Digestion. Water 9, w9110826.
Zhang, F.F., Wang, W., Yuan, S.J., Hu, Z.H., 2014. Biodegradation and speciation of roxarsone in an anaerobic granular sludge system and its impacts. J. Hazard. Mater. 279, 562–568.
Zhang, J., Cao, T., Tang, Z., Shen, Q., Zhao, F.J., 2015a. Arsenic methylation and volatilization by arsenite S-adenosylmethionine methyltransferase in *Pseudomonas alcaligenes* NBRC14159. Appl. Environ. Microb. 81 (8), 2852–2860.
Zhang, J., Zhao, S., Xu, Y., Zhou, W., Huang, K., Tang, Z., Zhao, F.J., 2017a. Nitrate stimulates anaerobic microbial arsenite oxidation in paddy soils. Environ. Sci. Technol. 51 (8), 4377–4386.
Zhang, S.Y., Su, J.Q., Sun, G.X., Yang, Y., Zhao, Y., Ding, J., Chen, Y.S., Shen, Y., Zhu, G., Rensing, C., Zhu, Y.G., 2017b. Land scale biogeography of arsenic biotransformation genes in estuarine wetland. Environ. Microbiol. 19, 2468–2482.
Zhang, S.Y., Zhao, F.J., Sun, G.X., Su, J.Q., Yang, X.R., Li, H., Zhu, Y.G., 2015b. Diversity and abundance of arsenic biotransformation genes in paddy soils from southern china. Environ. Sci. Technol. 49 (7), 4138–4146.
Zhang, Y.Y., Li, H., 2019. Energy recovery from wastewater treatment plants through sludge anaerobic digestion: effect of low-organic-content sludge. Environ. Sci. Pollu. R. 26, 30544–30553.
Zhao, F.J., Harris, E., Yan, J., Ma, J., Wu, L., Liu, W., McGrath, S.P., Zhou, J.Z., Zhu, Y.G., 2013. Arsenic methylation in soils and its relationship with microbial *arsM* abundance and diversity, and as speciation in rice. Environ. Sci. Technol. 47 (13), 7147–7154.