water

MDPI

Short Note

Arsenic Transformation in Swine Wastewater with Low-Arsenic Content during Anaerobic Digestion

Weiwei Zhai [1], Tianyue Qin [1], Ting Guo [1], Muhammad Imran Khan [2], Xianjin Tang [1,*] and Jianming Xu [1]

[1] Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Institute of Soil and Water Resources and Environmental Science, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China; zhaiweiwei2006@163.com (W.Z.); 13735540754@163.com (T.Q.); zihuanerbangt@163.com (T.G.); jmxu@zju.edu.cn (J.X.)

[2] Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan; khanimran1173@yahoo.com

* Correspondence: xianjin@zju.edu.cn; Tel.: +86-571-8898-2483; Fax: +86-571-8898-2069

Received: 17 September 2017; Accepted: 23 October 2017; Published: 27 October 2017

Abstract: In this study, the raw wastewater (RW), and effluents from the acidogenic phase (AP) and methanogenic phase (MP) in a swine wastewater treatment plant were collected to investigate the occurrence and transformation of arsenic (As), as well as the abundance of As metabolism genes during the anaerobic digestion (AD) process. The results showed that total concentrations of As generally decreased by 33–71% after AD. Further analysis showed that the As species of the dissolved fractions were present mainly as dimethylarsinic acid (DMA), with arsenite (As(III)) and arsenate (As(V)) as the minor species. Moreover, real-time PCR (qPCR) results showed that As metabolism genes (arsC, arsenate reduction gene; aioA, arsenite oxidation gene and arsM, arsenite methylation gene) were highly abundant, with arsM being predominant among the metabolism genes. This study provides reliable evidence on As biotransformation in swine wastewater treatment process, suggesting that AD could be a valuable treatment to mitigate the risk of As in wastewater.

Keywords: swine wastewater; anaerobic digestion; arsenic; transformation; metabolism genes

1. Introduction

With the rapid growth of the swine industry, pig farms discharge a large amount of wastewater, which usually contains not only nutrients, such as nitrogen and phosphorus, but also harmful components, such as antibiotics and heavy metals [1]. Organoarsenic additives have been extensively used in chicken and swine feeds. Most of the supplemented Arsenic (As) is excreted with the feces, further discharging into the wastewater through washing. It subsequently enters the soil through manure land application, eventually being biodegraded to the more toxic inorganic As [2]. Microbially-mediated As metabolic processes play a major role in As cycling, including arsenite (As(III)) oxidation, arsenate (As(V)) reduction, and As(III) methylation [3], as speciation, bioavailability, toxicity, environmental behavior, and their fate in the environment are changed through these processes. The widespread As detoxification by As(V) reductase (ArsC) is a pathway for microbial As(V) reduction. As(III) oxidation is catalyzed by As(III) oxidases, which are encoded by aioA genes (As(III) oxidation, previously named as aox/aso/aro) [4]. Moreover, As(III) can be methylated by microbes into various organic As species [5], which are catalyzed by the As(III) methylation (arsM) genes. These genes and their corresponding proteins are frequently used as molecular markers for microbial and ecological studies [6].

Anaerobic digestion (AD) is an important and effective treatment process for swine wastewater, which can reduce the energy expenditure, while producing renewable energy [1]. AD process optimizes

the conditions (pH, temperature) by separating the acidogenic phase (AP) and methanogenic phase (MP) to enhance the overall degradation efficiency [7]. The AD process influences the accumulation and distribution of heavy metals [8], which is a critical factor in predicting the mobility and eco-toxicity of heavy metals (especially As) [9]. During the AD treatment process, As can be transformed from stable fractions to more bioavailable/toxic fractions with the addition of water and decomposition. However, there are few reports on the transformations and metabolism genes of As in anaerobic digesters treating As-contaminated swine wastewater.

The aims of this study were to, (a) investigate the occurrence and behavior of the As when the swine wastewater was subjected to AD; and, (b) examine the distribution and abundance of As metabolism genes in swine wastewater treated by AD.

2. Materials and Methods

2.1. Swine Wastewater Treatment Process and Sample Collection

The swine wastewater treatment plant is located in Haiyan, Zhejiang Province, China. Successive treatment processes have been set to reduce the level of pollution from wastewater before discharging it to the environment. Firstly, the raw wastewater (RW) is retained in the regulating reservoir after solid-liquid separation. Secondly, the liquid fraction is delivered to a hydrolytic acidification tank reactor as AP. Lastly, the liquid fraction is carried to anaerobic digesters as MP, which is a plug-flow reactor. The waste influent enters the plug-flow reactor from AP, eventually exiting from the other end. A portion of the effluent is typically recycled to inoculate the influent, thus improving the AD. The hydrolytic acidification reactor and anaerobic digesters are both run at ambient temperature (10–35 °C), with a hydraulic retention time (HRT) of about 15 days. Feeding is processed at a rate ranging from 20 to 40 m^3 day^{-1}, depending on outputs of the farms. Biogas is collected for power generation, which is used for lighting and heating purposes at the farms. The RW, effluent after AP and MP were collected and analyzed in this study. Three subsamples were collected from each treatment. Samples were collected once every two months (April, June, and August).

2.2. Determination of Physico-Chemical Properties

Chemical oxygen demand (COD) was determined using a COD reactor (DRB200, Hach, Loveland, CO, USA), and ammonia nitrogen (N-NH_4^+) was determined using the colorimetric methods [10]. pH of the liquor was measured using a digital pH meter (Mettler, S220-Bio-CN, Giessen, Germany).

2.3. Total Heavy Metal and Dissolved Fractions of As Analysis

After digesting the water samples with nitric acid/hydrogen peroxide mixture, heavy metal concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS, NEXION300XX, PerkinElmer, Inc., Shelton, CT, USA). The dissolved fractions of As were (filtered through 0.45-µm membrane syringe filters ((Millipore, Bedford, MA, USA)) kept at −20 °C till further analysis. The concentrations of total As and different As species were measured as described previously [11].

2.4. Determination of Bacterial 16S rRNA and As Metabolism Genes Copies by qPCR

After passing water samples through 0.45-µm filters, DNA was extracted from the filters using an Ultraclean Water DNA Kit (MoBio Laboratories, Carlsbad, CA, USA), following manufacturer instructions. Concentration of the extracted DNA was estimated using spectrophotometer analysis (NanoDrop ND-2000, NanoDrop Co., Wilmington, DE, USA) and the extracted DNA was stored at −80 °C until further analysis. The arsC, aioA, arsM and 16S rRNA gene copy numbers were measured through qPCR analysis. The qPCR thermocycling conditions for 16S rRNA gene and As metabolism genes were described elsewhere [12].

3. Results and Discussion

3.1. Physico-Chemical Properties and Heavy Metals in Wastewater

pH is a parameter that greatly affects the AD process. The pH values 7.1–7.5 (Table 1) were within the permissible range (6.5–8.5) for AD [13]. The increase of pH in MP can be explained by the volatile fatty acids (VFA), converted to CH_4 and CO_2. In addition, biodegradable organic substances converted into methane and CO_2 resulting in COD decrease in the digester. The COD removal efficiency of the AP process was in the range of 3–13%, which increased to a range of 54–62% after MP. Kim et al. reported that COD was barely reduced in the acidogenic phase and the efficiency of COD reduction during the methanogenic HRT ranging between 25 and 10 d was between 67.0% and 63.7% [14]. Another previous study has reported that COD removal efficiencies ranged from 80% to 97% in the methanogenic reactors, indicating an effective biodegradation [15]. The NH_4^+-N concentrations of the effluents from the MP reactor systems were significantly higher than those of RW or AP, implying that most of the ammonium was generated in the MP reactor, which may be due to the mineralization of organic nitrogen. The total concentrations of Zn, Cu, Fe, Mn and Cd in MP were much lower than those in RW (Table 1). On the one hand, most of the heavy metals deposited in the bottom of the digester as associated with the suspended solids for a relatively long period [16]. On the other hand, the sulfide produced by sulfate reduction can react with many heavy metals, forming metal sulfides, which are insoluble and sediment rapidly [17], resulting in the decrease of heavy metals in the liquid matter.

Table 1. General chemical properties of swine wastewater samples of raw wastewater (RW), acidogenic phase (AP), and methanogenic phase (MP) in different months.

Month	Treatment	pH	COD Removal Efficiency (%)	NH_4^+-N (mg L^{-1})	As (μg L^{-1})	Cu (μg L^{-1})	Zn (μg L^{-1})	Fe (μg L^{-1})	Mn (μg L^{-1})	Cd (μg L^{-1})
April	RW	7.1 ± 0.1a	-	891.9 ± 3.1a	294.0 ± 19.1a	433.5 ± 101.0a	1647.3 ± 361.9a	2829.5 ± 1262.9a	400.3 ± 122.6a	0.3 ± 0.1a
	AP	7.1 ± 0.1a	3.2 ± 1.5	927.1 ± 40.5a	235.2 ± 102.8a	497.6 ± 34.3a	1100.3 ± 118.3b	2294.1 ± 1345.8a	374.5 ± 98.6a	0.4 ± 0.3a
	MP	7.5 ± 0.1b	60.8 ± 8.8	939.3 ± 30.6a	196.6 ± 16.9b	211.4 ± 5.3b	674.7 ± 19.0c	2947.3 ± 415.7a	187.4 ± 8.3b	0.2 ± 0.0a
June	RW	7.2 ± 0.1a	-	598.5 ± 5.5b	440.1 ± 46.7a	222.9 ± 1.7a	718.4 ± 7.9a	1052.3 ± 41.1a	174.8 ± 4.1a	0.2 ± 0.0a
	AP	7.1 ± 0.0a	10.9 ± 4.9	572.3 ± 26.0b	204.3 ± 10.7b	253.3 ± 5.3a	986.0 ± 12.6a	1523.6 ± 25.1b	196.3 ± 14.3a	0.2 ± 0.0a
	MP	7.4 ± 0.1b	61.9 ± 2.2	785.7 ± 41.4a	146.1 ± 32.6c	165.9 ± 41.4b	535.3 ± 45.9b	597.4 ± 162.8c	110.0 ± 29.3b	0.2 ± 0.1a
August	RW	7.1 ± 0.0a	-	346.0 ± 14.2c	208.3 ± 46.7a	268.9 ± 39.6a	865.5 ± 123.8a	1261.4 ± 180.3a	219.5 ± 47.4a	0.3 ± 0.1a
	AP	7.2 ± 0.0a	13.0 ± 3.2	315.7 ± 22.5c	83.2 ± 8.3b	100.5 ± 6.9b	306.2 ± 34.5b	549.2 ± 28.5b	121.9 ± 10.5b	0.3 ± 0.0a
	MP	7.3 ± 0.0a	53.6 ± 1.8	365.9 ± 49.2c	59.9 ± 5.2c	86.2 ± 17.2c	250.5 ± 25.1c	401.6 ± 93.9b	63.5 ± 19.5c	0.2 ± 0.0a

Note: Different lowercase letters in the columns indicate a significant difference among the different samples at $p < 0.05$.

3.2. Total As Changes during AD

In the present study, total concentrations of As in the samples were 208–440 μg L^{-1} (Table 1), which decreased (33.1–71.2%) in the samples of MP as compared to that in RW. The As removal was carried out by removal of solid forms through MP, as most of the As is associated with the suspended solids [8]. On the other hand, the effects of sampling time on total concentrations of As were obvious, which was caused by the variations in veterinary supplement and drug usage in different months. Jin and Chang reported that total concentrations of As in digested pig slurries were 20–100 μg L^{-1} [16]. Repeated and intense localized disposal of digested pig slurries can introduce a substantial amount of As into the environment, which should raise enough concerns on the heavy metal pollution, despite its fertilizer value.

3.3. Dissolved Fractions of As

Four As species, As(III), As(V), monomethylarsenic acid (MMA), and dimethylarsinic acid (DMA), measured in the dissolved fractions of swine water, accounted for 3.2–14.2% of the total concentrations (Figure 1). The proportion of dissolved fractions to total concentrations was lower than that reported by Jin and Chang [16]. Concentrations of the four species did not increase according to the increase of total concentrations in pig slurries, indicating that the chemical forms of As were not only dependent on contents, but also on the conditions of operation and storage. The As(III) content decreased by 58.1%, 5.4% and 51.3% in April, June and August during AP, while it decreased by 4.1%, 22.3%, 23.2% in April, June, and August during MP. The As(V) remained labile during those three months. The contents of DMA of RW were 18.6 ± 1.0, 12.0 ± 4.0, and 10.0 ± 1.1 μg L^{-1} in the samples from April, June, and August, followed by a rapid decrease of 63.9%, 36.2%, and 69.2% after MP, suggesting the potential loss of DMA. MMA was mostly detected in the sample of MP. This may be due to the presence of different As(V)-reducing microorganisms in the biogas slurry that transform As(V) to As(III) under reduction conditions, subsequently converting it to MMA, DMA, and trimethylarsine (TMA), which is released with the methane through As methylation [18]. MMA is the intermediate phase of methylation. During MP, a fraction of DMA may be reduced to dimethylarsine, or further methylated to form TMA, both of which are volatile. Mohapatra et al. reported that up to 35% of As can be volatilized from AD of cow dung using cultures of methanogenic bacteria [19].

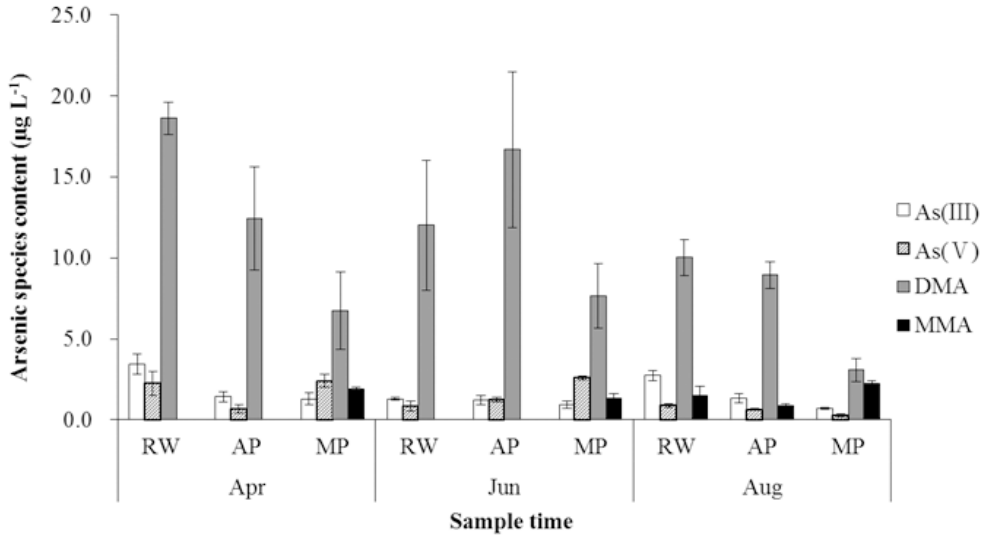

Figure 1. Dissolved (<0.45 μm) concentrations of different As species in the swine wastewater samples of raw wastewater (RW), acidogenic phase (AP) and methanogenic phase (MP) in different months.

3.4. Abundance of As Metabolism Genes

Transformation of the As species is greatly influenced by the microorganisms. The arsC, aioA, and arsM genes were detected and the absolute gene copy numbers were normalized to that of ambient 16S rRNA genes to minimize the variances caused by the differences in background bacterial abundances, extraction, and analytical efficiencies. The relative expression of functional genes in different samples showed significant differences. The arsC genes had a relative expression of $0.17-1.96 \times 10^{-7}$ (Figure 2a). The arsC genes are more widespread among both anaerobic and aerobic microbes [20,21]. A higher expression of the arsC gene was found in paddy soils, indicating that the microbes that reduce As(V) under aerobic conditions may be more abundant than the anaerobic microbes [22]. Subsequently, the expression of arsC decreased after AP and MP, as compared to RW. The arsC-mediated As(V) reduction process seems to be the most indispensable part of the As biogeochemical cycle. The expression of aioA ranged from 0.07×10^{-7} to 3.61×10^{-7} (Figure 2b). Since As(V) was less toxic than As(III), and As(V) is immobilized and more easily removed than As(III) from aqueous environments, As(III) oxidation is believed to be the microbial detoxification metabolism in various environments. There was no significant difference in the aioA gene copies between treatments every month, probably due to the decrease of mobile As(III) in AP and the anaerobic condition in MP. The abundance of arsM genes was almost 100 times higher ($0.10-3.61 \times 10^{-5}$; Figure 2c) than arsC genes and aioA genes in the samples, implying a strong potential to produce methylated As species. The highest expression of arsM could explain why most samples contain unusually high methylated As concentrations. Moreover, the expression of arsM was higher than those in AP and MP, since As methylation was stimulated under anaerobic conditions. During the AD of fresh pig manure with organic matter, microorganisms can volatilize As into AsH_3, $AsH_2(CH_3)$, $AsH(CH_3)_2$, and $As(CH_3)_3$, respectively [23]. The high abundance of redox and methylation genes suggested that high levels of microbial activities related to As metabolism may exist in the swine wastewater treatments.

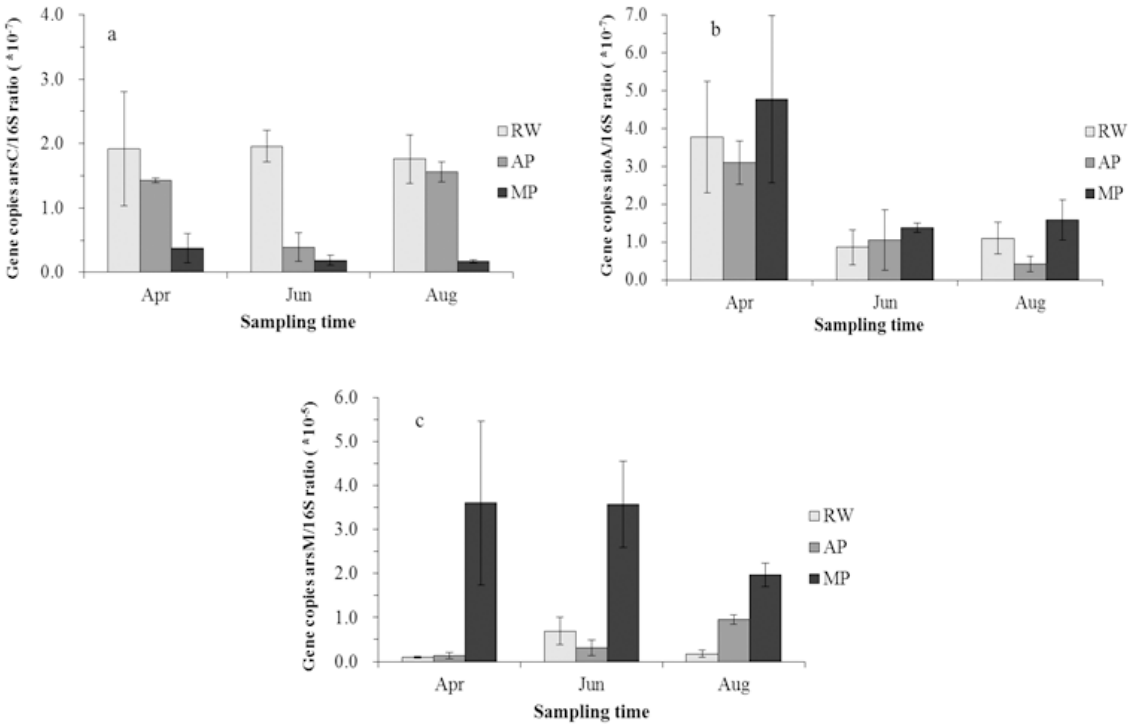

Figure 2. The abundance of As metabolism genes (**a**: arsC gene; **b**: aioA gene; **c**: arsM gene) in different samples of raw wastewater (RW), acidogenic phase (AP), and methanogenic phase (MP) in different months.

3.5. As Species Concentration vs. As Metabolism Gene Copy Numbers

A positive correlation (R^2 = 0.69, p = 0.005) was observed between arsC gene copy numbers and As(III) concentrations (Figure 3a). The abundance of arsC genes increased with an increasing As concentration. Moreover, arsC has been reported to be the dominant gene involved in the intracelluar microbial As detoxification process [24]. With the relatively high R^2 values for arsC, we strongly propose that the survival of the microbes detected under such conditions could be attributed to the As detoxification processes. There was a linear relationship between the abundance of aioA gene and the concentrations of As(V) (R^2 = 0.35, p = 0.096). However, the correlation was not significant (Figure 3b). The abundance of the arsM gene was significantly negatively correlated with the total methylated As contents (R^2 = 0.44, p = 0.052, Figure 3c). It can be explained by several possible reasons. For example, environmental conditions influence the As methylation activity of microbes, while the abundance of As methylation microbes has little effect on As methylation. A previous study reported that the arsM gene copy number correlated positively with the soil pH [25]. Although there were more As methylated microbes in neutral alkaline soils, the activity of As methylation was higher in acidic soils. Secondly, the decrease in DMA concentration after MP reflected the loss of methylated As through either volatilization or demethylation. As volatilization can be mediated through the enzymes that are involved in methanogenesis. It has been reported that a range of arsine and methyl-arsine species from arsenate were produced by cultures of methanogens [26]. The impact of methanogenesis on As volatilization has been studied in As-bearing ferric iron waste, in which As volatilization represented <0.02% of the total As added [18]. The quantification of genes involved in As biotransformation processes can be taken as an indicator for As transformation. The linkage between the As metabolism gene and the As species implied that the As transformation in the AD plant should be taken into consideration.

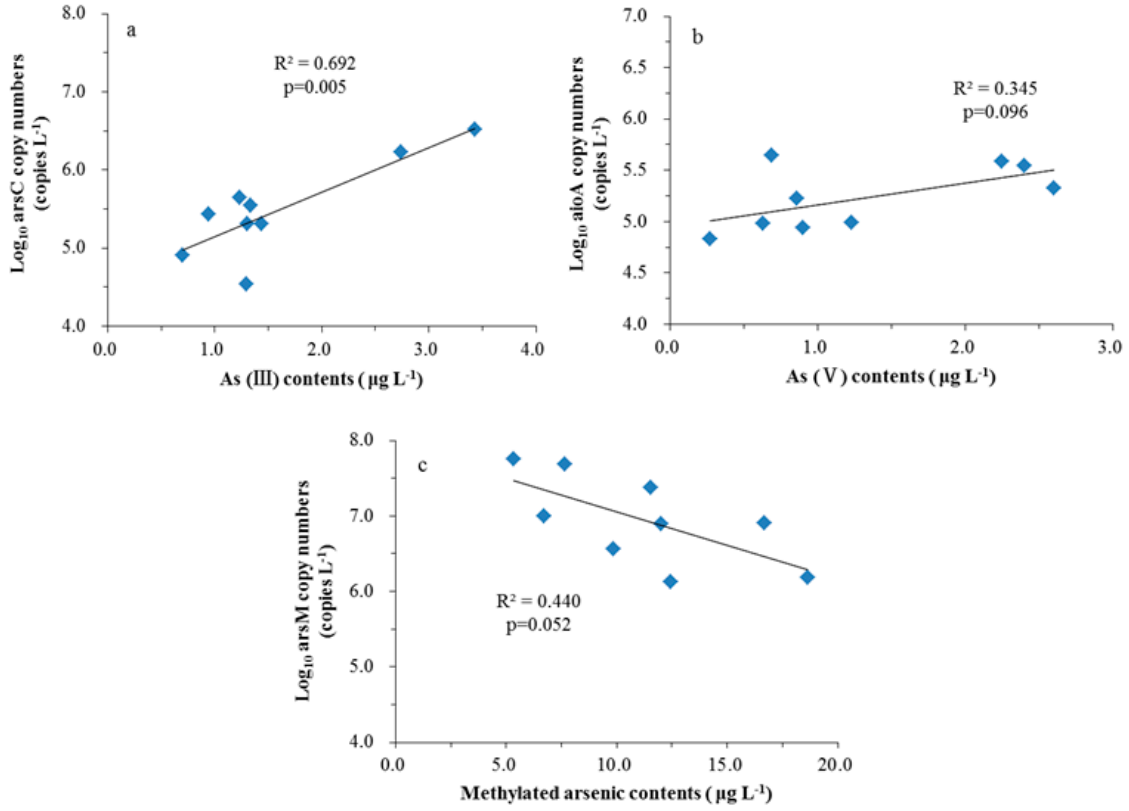

Figure 3. Correlation analyses between different As species concentration vs. As metabolism gene copy numbers. (**a**: As(III) contents vs. arsC gene; **b**: As(V) contents vs. aioA gene; **c**: Methylated As contents vs. arsM gene).

4. Conclusions

The results from this study show that the total concentrations of As decreased by 33–71% after AD, As species of the dissolved fractions was present mainly as DMA. Specially, using qPCR, it showed that As metabolism genes were abundant and universal even in low-As environments. There were correlations between the abundance of As detoxification genes and As contents, which prove that the transformations of As based on different As resistance systems of microbes during the wastewater treatment process. However, further studies should be carried out to define the functional microbes contributing to As transformation during AD of swine wastewater.

Acknowledgments: This work was supported by the Fundamental Research Funds for the Central Universities and the National Science and Technology Support Program (2014BAD14B04).

Author Contributions: Weiwei Zhai conducted the experiments and wrote the main part of the manuscript. Tianyue Qin processed and performed analysis of data. Ting Guo helped to perform the analysis of data and revised the manuscript. Muhammad Imran Khan revised the manuscript. Xianjin Tang conceived and designed the experiments and revised the manuscript. Jianming Xu also revised the manuscript. All authors approved the final version of the manuscript.

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

References

1. Do Amaral, A.C.; Kunz, A.; Radis Steinmetz, R.L.; Justi, K.C. Zinc and copper distribution in swine wastewater treated by anaerobic digestion. *J. Environ. Manag.* **2014**, *141*, 132–137. [CrossRef] [PubMed]
2. Fisher, D.J.; Yonkos, L.T.; Staver, K.W. Environmental Concerns of Roxarsone in Broiler Poultry Feed and Litter in Maryland, USA. *Environ. Sci. Technol.* **2015**, *49*, 1999–2012. [CrossRef] [PubMed]
3. Meharg, A.A.; Zhao, F.J. Biogeochemistry of arsenic in paddy environments. In *Arsenic & Rice*; Springer Science & Business Media Press: Dordrecht, The Netherlands, 2012; pp. 71–101.
4. Lett, M.C.; Muller, D.; Lievremont, D.; Silver, S.; Santini, J. Unified Nomenclature for Genes Involved in Prokaryotic Aerobic Arsenite Oxidation. *J. Bacteriol.* **2011**, *194*, 207–208. [CrossRef] [PubMed]
5. Qin, J.; Rosen, B.P.; Zhang, Y.; Wang, G.; Franke, S.; Rensing, C. Arsenic detoxification and evolution of trimethylarsine gas by a microbial arsenite S-adenosylmethionine methyltransferase. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2075–2080. [CrossRef] [PubMed]
6. Lever, M.A. Functional gene surveys from ocean drilling expeditions—A review and perspective. *FEMS Microbiol. Ecol.* **2013**, *84*, 1–23. [CrossRef] [PubMed]
7. Zhao, Y.; Wu, J.; Yuan, X.; Zhu, W.; Wang, X.; Cheng, X.; Cui, Z. The effect of mixing intensity on the performance and microbial dynamics of a single vertical reactor integrating acidogenic and methanogenic phases in lignocellulosic biomass digestion. *Bioresour. Technol.* **2017**, *238*, 542–551. [CrossRef] [PubMed]
8. Marcato, C.E.; Pinelli, E.; Pouech, P.; Winterton, P.; Guiresse, M. Particle size and metal distributions in anaerobically digested pig slurry. *Bioresour. Technol.* **2008**, *99*, 2340–2348. [CrossRef] [PubMed]
9. Garbarino, J.R.; Bednar, A.J.; Rutherford, D.W.; Beyer, R.S.; Wershaw, R.L. Environmental Fate of Roxarsone in Poultry Litter. I. Degradation of Roxarsone during Composting. *Environ. Sci. Technol.* **2003**, *37*, 1509–1514. [PubMed]
10. APH Association. *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; APH Association: Washington, DC, USA, 1998; p. 1268. Available online: https://www.mwa.co.th/download/file_upload/SMWW_1000-3000.pdf (accessed on 24 October 2017).
11. Zhai, W.; Wong, M.T.; Luo, F.; Hashmi, M.Z.; Liu, X.; Edwards, E.A.; Tang, X.; Xu, J. Arsenic Methylation and its Relationship to Abundance and Diversity of arsM Genes in Composting Manure. *Sci. Rep.* **2017**, *7*, 42198. [CrossRef] [PubMed]
12. Zhang, S.; Zhao, F.; Sun, G.; Su, J.; Yang, X.; Li, H.; Zhu, Y. Diversity and Abundance of Arsenic Biotransformation Genes in Paddy Soils from Southern China. *Environ. Sci. Technol.* **2015**, *49*, 4138–4146. [CrossRef] [PubMed]
13. Dong, B.; Liu, X.; Dai, L.; Dai, X. Changes of heavy metal speciation during high-solid anaerobic digestion of sewage sludge. *Bioresour. Technol.* **2013**, *131*, 152–158. [CrossRef] [PubMed]

14. Kim, W.; Shin, S.G.; Cho, K.; Lee, C.; Hwang, S. Performance of methanogenic reactors in temperature phased two-stage anaerobic digestion of swine wastewater. *J. Biosci. Bioeng.* **2012**, *114*, 635–639. [CrossRef] [PubMed]
15. Parawira, W.; Murto, M.; Read, J.S.; Mattiasson, B. Profile of hydrolases and biogas production during two-stage mesophilic anaerobic digestion of solid potato waste. *Process Biochem.* **2005**, *40*, 2945–2952. [CrossRef]
16. Jin, H.; Chang, Z. Distribution of Heavy Metal Contents and Chemical Fractions in Anaerobically Digested Manure Slurry. *Appl. Biochem. Biotechnol.* **2011**, *164*, 268–282. [CrossRef] [PubMed]
17. Paulo, L.M.; Stams, A.J.; Sousa, D.Z. Methanogens, sulphate and heavy metals: A complex system. *Rev. Environ. Sci. Biotechnol.* **2015**, *14*, 537–553. [CrossRef]
18. Webster, T.M.; Reddy, R.R.; Tan, J.Y.; Van Nostr, J.D.; Zhou, J.; Hayes, K.F.; Raskin, L. Anaerobic Disposal of Arsenic-Bearing Wastes Results in Low Microbially Mediated Arsenic Volatilization. *Environ. Sci. Technol.* **2016**, *50*, 10951–10959. [CrossRef] [PubMed]
19. Mohapatra, D.; Mishra, D.; Chaudhury, G.R.; Das, R.P. Removal of Arsenic from Arsenic Rich Sludge by Volatilization using Anaerobic Microorganisms Treated with Cow Dung. *Soil Sediment Contam.* **2008**, *17*, 301–311.
20. Bhattacharjee, H.; Rosen, B.P. Arsenic metabolism in prokaryotic and eukaryotic microbes. In *Molecular Microbiology of Heavy Metals*; Springer: Berlin/Heidelberg, Germany, 2007; pp. 371–406.
21. Silver, S.; Phung, L.T. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Appl. Environ. Microbiol.* **2005**, *71*, 599–608. [CrossRef] [PubMed]
22. Luo, J.; Bai, Y.; Liang, J.; Qu, J. Metagenomic approach reveals variation of microbes with arsenic and antimony metabolism genes from highly contaminated soil. *PLoS ONE* **2014**, *9*, e108185. [CrossRef] [PubMed]
23. Mestrot, A.; Merle, J.K.; Broglia, A.; Feldmann, J.; Krupp, E.M. Atmospheric Stability of Arsine and Methylarsines. *Environ. Sci. Technol.* **2011**, *45*, 4010–4015. [CrossRef] [PubMed]
24. Martin, P.; DeMel, S.; Shi, J.; Gladysheva, T.; Gatti, D.L.; Rosen, B.P.; Edwards, B.F. Insights into the structure, solvation, and mechanism of ArsC arsenate reductase, a novel arsenic detoxification enzyme. *Structure* **2001**, *9*, 1071–1081. [CrossRef]
25. Zhao, F.; Harris, E.; Yan, J.; Ma, J.; Wu, L.; Liu, W.; McGrath, S.P.; Zhou, J.; Zhu, Y. Arsenic Methylation in Soils and Its Relationship with Microbial arsM Abundance and Diversity, and As Speciation in Rice. *Environ. Sci. Technol.* **2013**, *47*, 7147–7154.
26. Meyer, J.; Michalke, K.; Kouril, T.; Hensel, R. Volatilisation of metals and metalloids: An inherent feature of methanoarchaea? *Syst. Appl. Microbiol.* **2008**, *31*, 81–87. [CrossRef] [PubMed]