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Linkages of volatile fatty acids and polyhexamethylene guanidine stress during sludge fermentation: Metagenomic insights of microbial metabolic traits and adaptation

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ABSTRACT

The massive use of polyhexamethylene guanidine (PHMG), as a typical bactericidal agent, raised environmental concerns to the public. This work comprehensively revealed the hormesis effects of PHMG occurred in waste activated sludge (WAS) on the generation of volatile fatty acids (VFAs) during anaerobic fermentation. The low level of PHMG (100 mg/g TSS) significantly promoted the VFAs generation (1283 mg COD/L, compared with 337 mg COD/L in the control) *via* synchronously facilitating the solubilization, hydrolysis, and acidification steps but inhibiting methanogenesis. Metagenomic analysis showed that the functional anaerobe (*i.e.*, *Bacteroides*, *Macellibacteroide* and *Parabacteroide*) and corresponding genetic expressions responsible for extracellular hydrolysis (*i.e.*, *clpP*), membrane transport (*i.e.*, *ffh* and *gspF*), intracellular substrates metabolism (*i.e.*, *ald* and *paaF*) and VFAs biosynthesis (*i.e.*, *ACACA* and *FASN*) were enhanced in the optimal presence of PHMG. Moreover, the anaerobic species could respond and adapt to low PHMG stimuli *via* quorum sensing (*i.e.*, *cqsA*, *rpfC* and *rpfG*), and thus maintain the high microbial metabolic activities. However, they were unable to tolerate the toxicity of excessive PHMG, resulting in the extremely low VFAs production. This work enlightened the effects of emerging pollutants on WAS fermentation at the genetic levels, and provided guidance on the WAS treatment and resource recovery.

Due to the growing populations and urbanization acceleration, the effective handling of environmental issues caused by waste activated sludge (WAS) is a huge challenge for wastewater treatment plants (WWTP) in populated urban areas [1,2]. It has been estimated that 11.2 million tons/year of dry sludge were generated in China [3], and the costs for its effective disposal have accounted for up to 30%–60% of the overall operation cost of WWTPs. Inspiringly, WAS contains high levels of organic compounds, which could be utilized as an ideal renewable resource repository [4]. As an eco-friendly technology, anaerobic fermentation is frequently applied to disposal of WAS for achieving volume reduction and bioresource production (*i.e.*, volatile fatty acids (VFAs)) [5,6]. It is well-known that VFAs (particularly acetic and propionic acids) are valuable carbon sources with high economic value and multifaceted applications, whose market prices are approximately 800–2100 €/tonnes [7].

However, the WAS also contains various undesirable pollutants, which may cause environmental risks and affect the subsequently treatment efficiency [8]. Among them, polyhexamethylene guanidine (PHMG) is widely used as a bactericidal agent, and has been conducted as the active ingredient in a variety of wipes, disinfectants, and wound rinse disinfectants [9]. Obviously, the largely consumed PHMG would enter into wastewater and eventually accumulate in WAS, which exhibits potential impacts on the subsequent biological treatment. However, up to now, the PHMG impacts on the WAS anaerobic fermentation for VFAs accumulation remains unclear. Meanwhile, how dose the PHMG influence the specific fermentation process (*i.e.*, solubilization, hydrolysis, acidification and methanogenesis) is still an open question. Furthermore, various functional microorganisms and the corresponding genes are involved in biochemical reactions of VFAs generation [10]. It is unclear how these anaerobes and functional genes change in response to PHMG stress during the WAS fermentation process.

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Besides, as an antimicrobial agent, PHMG also exhibits certain toxicity to microorganisms and affects the biological metabolism [11]. Whether those functional anaerobic species enable to adapt to the unfavorable conditions induced by PHMG and how to maintain the high microbial activities for VFAs production require further explorations.

Hence, this study investigated the potential impacts of PHMG on the WAS fermentation for VFAs production, and disclosed the underlying mechanism in view of the microbial metabolic traits and adaptive mechanisms *via* metagenomic analysis. Firstly, the dose-dependent impacts of PHMG on VFAs yields and particular stages were determined. Then, the variation of microorganisms and the metabolic pathway that participated in VFAs production in response to PHMG stress were studied. Finally, the mechanisms of microbial adaption to PHMG stimulus were unveiled. This work enlightened the emerging pollutants impacts on WAS fermentation at the genetic levels, and provided guidance on the WAS treatment and resource recovery.

The WAS was obtained from the WWTP in Nanjing, China (A^2/O, HRT (hydraulic retention time): 17.5 h), and then filtered to eliminate those undesirable inorganics (2.0 mm) and naturally concentrated over 24 h at 4 °C. The main WAS properties were as follows: pH 7.01 ± 0.01, total suspended solids (TSS) 19.95 ± 0.02 g/L, volatile suspended solids (VSS) 9.55 ± 0.05 g/L, total chemical oxygen demand (TCOD) 21,050 ± 130 mg/L, soluble chemical oxygen demand (SCOD) 422 ± 8 mg/L, total proteins 6.2 ± 0.2 g/L, total carbohydrates 1.7 ± 0.3 g/L. Meanwhile, the PHMG with a purity of ≥95% was bought from Sigma-Aldrich (St. Louis, MO, USA).

Identical serum bottles (600 mL working volume) were applied for batch fermentation tests, and all tests were conducted in triplicates. 300 mL pretreated WAS was fed into each reactor firstly, then the reactor with no PHMG dose were kept as the control while the other groups were added with 100 (low dose) and 400 (high dose) mg/g TSS PHMG. After being purged with N_2 for 5 min to maintain the anaerobic environment, all systems were sealed with butyl rubbers immediately, and then placed in an air-bath shaker (180 rpm) under 37.5 ± 1 °C. The samples were withdrawn every 2 d, and then centrifugated and filtered to obtain the supernatant for VFAs detection.

Moreover, to reveal the mechanism of VFAs production by PHMG, the levels of soluble carbohydrate and protein (indicate the solubilization efficiency), the content of NH_4^+-N and PO_4^{3-}-P (indicate hydrolysis efficiency), and the concentration of accumulative methane (indicate methanogenesis efficiency) were determined. Also, the adenosine 5′-triphosphate (ATP) was measured at 2 d to determine general microbial activity. Finally, 50 mL sludge mixtures were obtained at 10 d and stored immediately at −80 °C for further metagenomic analysis.

The TSS, VSS, TCOD, SCOD, NH_4^+-N, and PO_4^{3-}-P were examined based on the standard method [12]. The concentration of soluble carbohydrates and proteins were determined by the phenol-sulfuric and Lowry-Folin methods as the standard, respectively. The VFAs and methane yield were measured through the gas chromatograph Agilent 7820A and Agilent 8890, respectively. The ATP level was tested by ATP assay kit (Beyotime, China) according to manufactures instructions.

Microbial DNA was processed by E.Z.N.A.® stool DNA Kit (Omega Bio-tek, Norcross, GA, USA). The metagenomic sequencing were constructed and sequenced at Shanghai Biozeron Biological Technology Co. Ltd. The specific functions and pathways were analyzed by using the pathways mapped by genes annotated in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/ or http://www.kegg.jp/).

Fig. 1A shown the total VFAs yield from WAS anaerobic fermentation with different levels of PHMG. The maximal VFAs concentration was merely approximately 337 mg COD/L at 4 d in the control reactor. However, it improved to 1283 mg COD/L with low dosage of PHMG, while it decreased to 164 mg COD/L with high dosage of PHMG. In addition, the composition of generated VFAs also changed (Fig. 1B). The main VFAs compositions in the control were mainly acetic and propionic acids, with the proportion of 32.8% and 37.9%, respectively. However, with the PHMG occurrence, the proportion of acetic acid was increased while that of propionic acid was reduced. The average ratio of acetic acid and propionic acid were 47.9% and 26.1%, and 74.8% and 25.2% in the presence of low and high PHMG, respectively. Commonly, acetic acid exhibits the largest market size (3500,00 ton/year), which is the preferred carbon source in WWTPs and important precursors for methanogenesis [13]. Overall, the low PHMG stimulated the VFAs production process, and contributed to the economic and practical values of generated products.

The efficient solublization and hydrolysis of organic matters (*i.e.*, carbohydrates and proteins) derived from WAS are critical to the VFAs production [14]. As exhibited in Fig. 1C, PHMG greatly

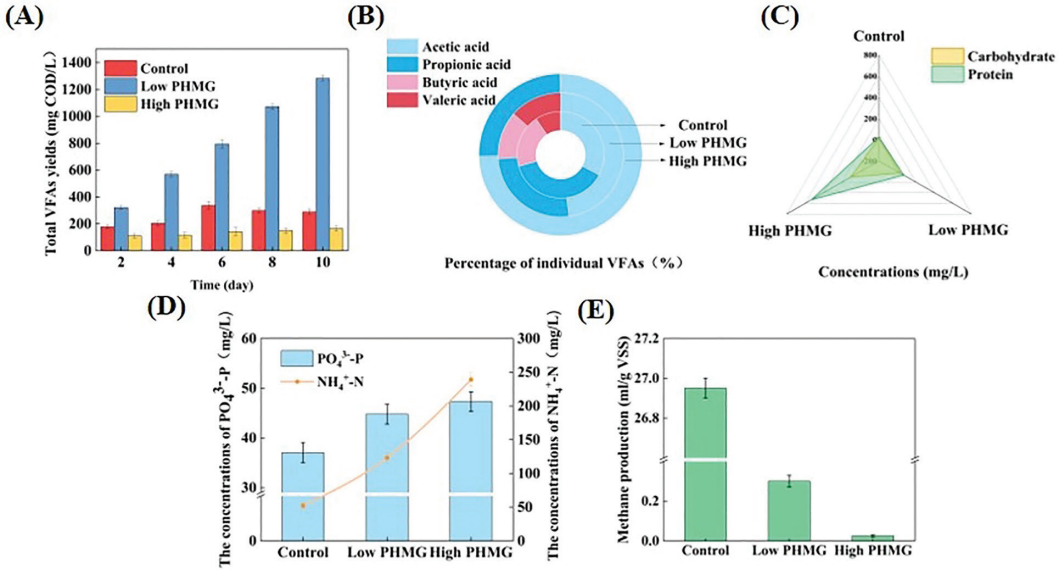

Fig. 1. Influence of PHMG dosage on the VFAs (A) generation, (B) composition during WAS fermentation, the concentration of (C) soluble carbohydrates and proteins, (D) NH_4^+ and PO_4^{3-}, and (E) cumulative methane yields in different reactors.

improved the solubilization process, and the promoting effects were more efficient on the releasement of proteins than carbohydrates. For example, the soluble proteins and carbohydrates were respectively 25.3 and 17.1 mg/L in the control reactor at 2 d. However, it increased to 68.0 and 28.4 mg/L with low PHMG, and further improved to 533.1 and 103.5 mg/L in high PHMG reactor. Moreover, the soluble NH_4^+-N and PO_4^{3-}-P derived from the organics hydrolysis in the WAS fermentation systems [15], were also observed to increase from 52.4 mg/L to 37.1 mg/L the in control reactor to 123.1 and 44.8 mg/L with low PHMG, and further elevated to 239.2 and 47.3 in high-PHMG dosed reactor (Fig. 1D). Obviously, the presence of PHMG efficiently promoted the solubilization and hydrolysis steps, which might provide more bioaccessible substrates to microbial metabolism for VFAs production.

Furthermore, the VFAs accumulation in WAS anaerobic fermentation was also highly related with the methanogenesis, which consumed the VFAs largely. As illustrated in Fig. 1E, the content of accumulative methane was 27.00 mL/g VSS in the control, while it gradually decreased to 0.30 and 0.03 mL/g VSS in the low- and high-dose PHMG reactors, respectively. These results unveiled that the presence of PHMG obviously inhibited the methane production, which could also contribute to the VFAs accumulation during WAS fermentation.

The metagenomic analysis on the critical microbial functions related to substrates metabolism and VFAs biosynthesis indicated that the expression levels of corresponding functional genes were generally upregulated in the presence of low PHMG, but they showed downward trends with excessive PHMG (Fig. 2). For instance, in terms of extracellular hydrolysis, the relative abundance of *clpP*, which was linked with the hydrolysis of amino acids by acting on peptide bonds (peptidases), was 1.87‰, 2.06‰, and 1.67‰ in the control, low and high PHMG reactors. However, the *celA*, which was related to the hydrolysis of polysaccharides, was negatively affected by the PHMG. These results were consistent with the more hydrolyzed protein substrates induced by PHMG. Similar results were also found for those transporters encoding genes, which were responsible for the deliver those extracellular hydrolysis products into intracellular cells for final metabolism [16,17]. For instance, in comparison with the control, the relative abundance of gene expressions involved in the amino acid metabolites delivery (*e.g.*, *ffh* and *gspF*), were enhanced by 1.1- and 1.1-fold in low PHMG reactor, but decreased by 0.9- and 0.8-fold with high dosage of PHMG, respectively.

Regarding the intercellular metabolism of fermentation substrates for VFAs production, the effects of PHMG on the functional genes (*i.e.*, *celF*, *pgi1*, *fbp3*, *ENO*) related to carbohydrates metabolism were insignificant. For example, the relative abundance of *ENO*, which participated in phosphoenolpyruvate formation, was 2.05‰, 2.06‰, and 2.07‰ in control, low, and high PHMG reactors, respectively. However, the critical genes expressions associated with proteins metabolism (*i.e.*, *aspB*, *gdha*, *ald*, *paaF*, *bkdA*, *bkdB*, *HADHB*, *vorB*, *ACADM*), were increased by 2%–98% in low PHMG reactor, while they were decreased by 1%–54% with high dosage of PHMG. Specifically, the relative abundance of *ACADM*, which was correlated with valine, leucine, and isoleucine degradation, was 2.06‰, 2.51‰, and 2.02‰ in control, low, and high PHMG reactors, respectively. Also, the genetic expression of *paaF* encoding for enoyl-CoA hydratase was 1.66‰, 2.13‰, and 1.65‰ in control, low, and high PHMG reactors, respectively.

Furthermore, the pyruvate metabolism, which was located in the central metabolic pathway and played essential roles in the final production of VFAs [10], was also affected by the presence of PHMG. The relative abundance of *ALDH7A1* encoding aldehyde dehydrogenase was enhanced by 1.6 folds in low PHMG reactor, while it was reduced by 0.5 folds in high PHMG reactor. Similar results were also found for other critical genes, such as *acs*, whose relative abundance was 0.72‰, 0.78‰, and 0.58‰ in control, low, and high PHMG reactors. Meanwhile, the gene expressions of *fadD*, *fabG*, *ACACA*, and *FASN* that were related to fatty acid biosynthesis, were observed to be enhanced by 3%–11% under low concentration PHMG compared with that of the control. Also, the relative abundance of *pta*, which was encoding for the vital enzymes regulating acetate production, was respectively 0.33‰ and 0.36‰ in control reactor and low PHMG reactor, while it was reduced to 0.19‰ with high dosage of PHMG. Obviously, the low level of PHMG induced the highly expressed genes, which were beneficial to catalyzing the synthesis and secretion of functional enzymes related to VFAs biosynthesis. But it was not the case for WAS fermentation system with high PHMG, which might be one of the primary reasons for the low VFAs generation efficiency despite of the high bioaccessible substrates.

In contrast, the metabolic pathways of acetoclastic methanogenesis were significantly reduced in the presence of PHMG as expected. The relative abundances of *mvhG* and *mfnBF*, which were involved in the synthesis of coenzymes F420, were reduced by 0.4–0.9 times in the reactor with low PHMG in comparison with the control. Moreover, the *cdhD* and *cdhE* that transformed the acetyl-CoA into 5-methyl-THMPT, and *mtrA* and *mtrC* encoding for the formation of Methyl-CoM, which were critical to the ultimate methane production [18], also showed downward trends (decreased by approximately 10%–66%) in the reactors with PHMG addition. Therefore, the downregulation of these vital genes implied

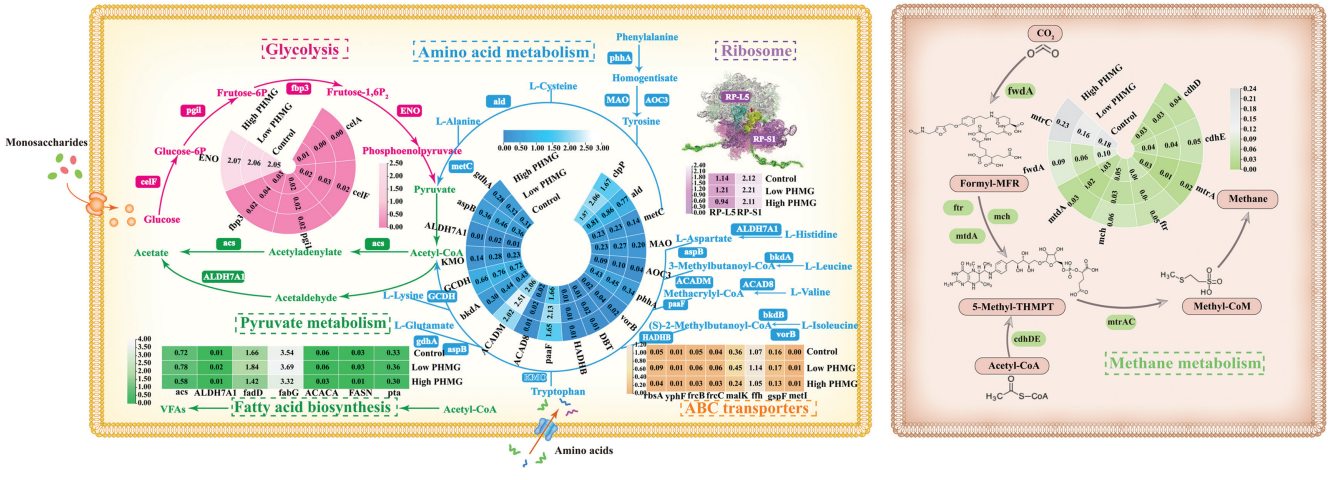

Fig. 2. Relative abundances of key encoding genes for critical metabolism pathways involved in VFAs synthesis.

the inhibition of methanogenic activity, which was also beneficial to the VFAs accumulation with less consumption.

Overall, the low dosage of PHMG contributed to the upregulation of genetic expression levels involved in extracellular hydrolysis, membrane transport, and intracellular substrate metabolism, while inhibiting the methane metabolism, thereby facilitating the ultimate VFAs generation.

The functional anaerobic species are the crucial players in the biochemical process for VFAs generation. As shown in Fig. 3A, the predominant bacterial phylum, including *Proteobacteria* (40.8%–50.8%), *Chloroflexi* (12.7%–13.6%), *Acidobacteria* (6.3%–8.8%), *Actinobacteria* (5.8%–13.8%), *Nitrospirae* (2.3%–5.7%), and *Bacteroidetes* (5.0%–8.0%), were observed in all reactors. They were usually considered as hydrolytic fermentation bacteria, and were related to organic decomposition and VFAs formation [19,20]. But the presence of PHMG affected their microbial composition evidently. For example, the abundance of *Actinobacteria*, which was positively correlated with VFAs production, was respectively 7.4% in the control, while it improved to 13.8% in low PHMG reactor, but decreased to 5.8% in high PHMG reactor. Additionally, the relative abundance of *Bacteroidetes*, which was known as proteolytic bacteria and responsible for protein decomposition [21], was 7.1%, 8.4%, and 5.8% in the presence of control, low- and high-dosage PHMG, respectively.

In terms of genus level (Fig. 3B), the functional microorganisms related to organic matters degradation and VFAs formation was also promoted with the stimulus of low PHMG while restrained at high levels. The relative abundances of *Alphaproteobacteria* sp. and *Proteiniborus*, which were involved in the metabolism of organic substances (especially proteins) [22–24], were 1.1- and 1.7-, and 0.7- and 1.9-fold in low and high PHMG reactors compared with the control group, respectively. Meanwhile, *Bacteroides*, *Macellibacteroide*, and *Parabacteroide*, which were able to convert protein compounds into acetate [24,25], were enriched by 2.8, 1.1 and 1.2 times with low PHMG compared with the control reactor, respectively. This might be ascribed to the stimulus of evident increase of bioavailable proteins as fermenation substrates in the corresponding reactors. However, they were respectively reduced by 0.6, 0.4, and 0.5 times in high PHMG reactor. This might be associated with the toxicity of excessive PHMG to the functional microorganisms. Moreover, the relative abundance of *Candidatus Accumulibacter* was found to be 1.9%, 1.1%, and 4.8% in control, low, and high PHMG reactors, respectively. The previous studies demonstrated that the *Candidatus Accumulibacter* was capable of utilizing VFAs and other

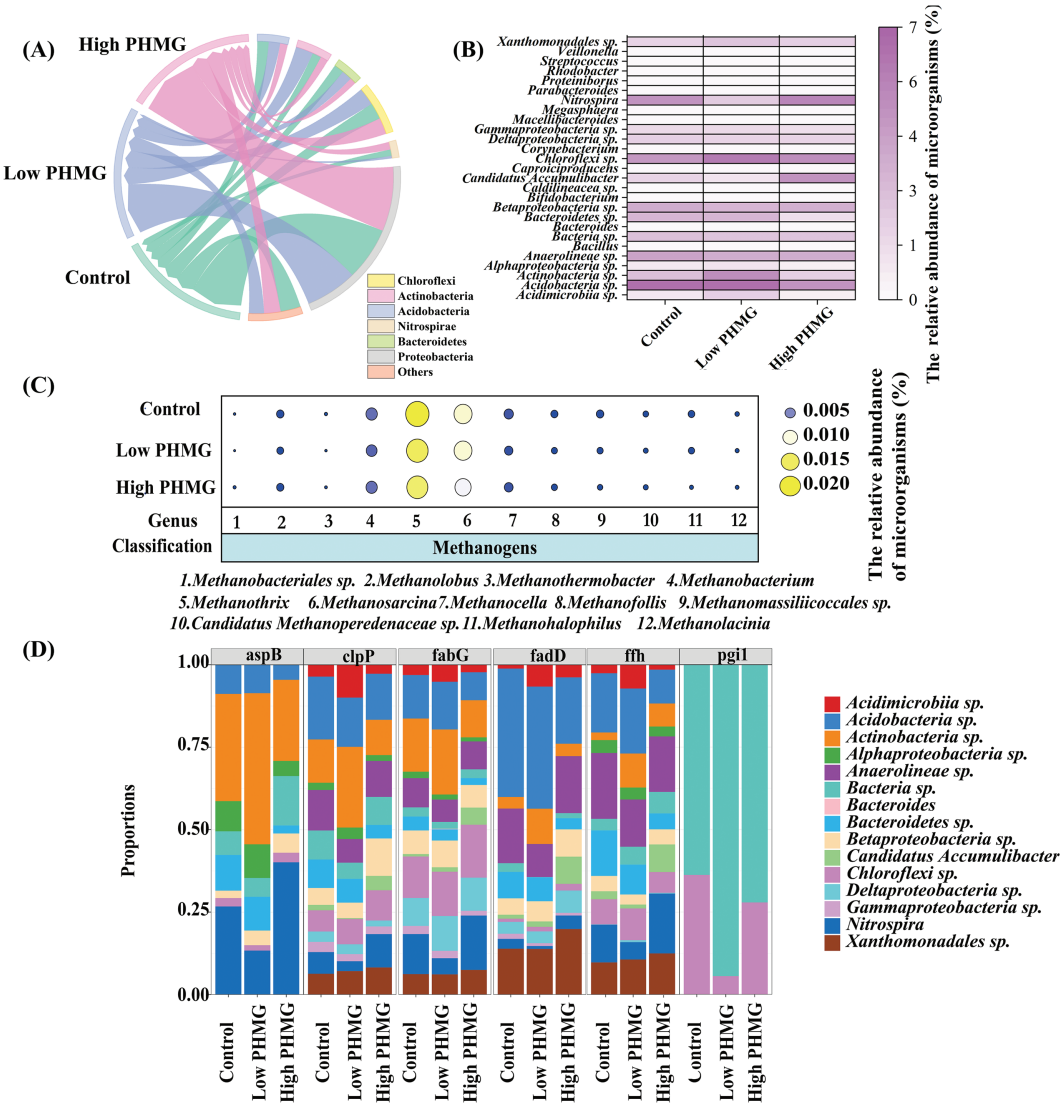

Fig. 3. Variation of main microbes at (A) phylum level, (B) genus level for bacteria, (C) genus level for methanogens in different reactors, and (D) microbial contribution analysis of the typical functional genes for VFAs formation.

intermediate compounds as carbon sources [26]. The reduction of these VFAs consumers was also beneficial to the final VFAs accumulation.

Regarding the typical methanogens, their abundances were evidently reduced in the presence of PHMG (Fig. 3C). For instance, the relative abundance of *Methanobacteriales* sp. and *Methanimicrococcus*, which were widely observed methanogens in the anaerobic systems [27], were respectively reduced by 0.7 and 0.2, and 1.6 and 0.8 times in the corresponding reactor with low and high PHMG reactors when comparing with those in control. These results were consistent with the significant methane reduction in PHMG-conditioned reactors as mentioned above.

Besides, the interrelationships between functional genetic expressions and fermentative microorganisms were further identified. Generally, the microbial contributions of key genes were enhanced in the reactor with low level of PHMG, while reduced in the high dosage of PHMG. As shown in Fig. 3D, the *clpP* involved in the hydrolysis step was linked to *Alphaproteobacteria* sp., and the contribution was 2.9%, 5.0%, and 1.8% in control, low and high PHMG reactors, respectively. Also, the *ffh*, which played a key role in membrane transport of fermentation substrates into microorganisms, was mainly linked to *Acidobacteria sp*., accounting for 13.5%, 16.7%, and 7.4% in control, low and high PHMG reactors. The microbial contribution of *aspB*, which was critical for amino acid metabolism, was mainly sourced from *Actinobacteria* sp. in control (9.8%) and low PHMG reactor (15.5%), while it was decreased in high PHMG reactor (6.9%). Besides, the *fadD* and *fabG*, which were essential genes for pyruvate metabolism, were expressed by *Acidobacteria* sp. (40.3% and 30.4% in control, 47.5% and 37.6% in low PHMG, and 16.6% and 19.6% in high PHMG, respectively) and *Actinobacteria* sp. (3.6% and 41.4% in control reactor, 13.7% and 51.2% in low PHMG reactor, and 3.1% and 26.1% in high PHMG reactor, respectively). Thus, the microbial populations were significantly affected by PHMG, which caused the differential genetic expressions responsible for the secretion of various metabolic enzymes, resulting in the dissimilarity of ultimate VFAs production.

As illustrated above, the low level of PHMG promoted VFAs during WAS fermentation, but the high dose of PHMG exhibited evident toxicity to the microorganisms and reduced the VFAs production significantly.

Previous publications proved that the microorganisms were able to adapt to certain toxic environments and resist the adverse effects of pollutants by other compensations, such as the increasing bioavailable substrates in this study [28]. The anaerobic species might be capable of adapting to the low concentrations of PHMG but collapsed at high dose. The analysis of metabolic functions based on KEGG classification demonstrated that the relative abundances of pathways involved in microbial adaptation were generally enhanced in low PHMG reactor (Fig. 4A). For instance, the relative abundances of genes related to cell growth and death were improved by 2.3% in low PHMG compared with that of control. On the contrary, it was respectively reduced by 5.6% in high PHMG reactor, indicating the dose-dependent toxicity of PHMG towards microorganisms. Similarly, the relative abundance of translation, which could translate mRNA during protein biosynthesis to maintain cell homeostasis and survival [29], was enhanced by 0.4% with low dosage of PHMG, while reduced by 11.2% with high dosage of PHMG. Correspondingly, the genetic expressions of *RPL5* and *RPS1* (Fig. 4B), which were involved in chaperones of the 5S rRNA and the small ribosomal subunit [30], were improved by 1.1 and 1.1 times in low PHMG compared with the control, while they were decreased by 0.8 and 0.9 times in high PHMG reactor.

The metabolism of quorum sensing was an effective cellular communication mechanism for microorganisms to regulate their community behavior. The microbial cells could effectively regulate transcription and activation of functional proteins and migrate microbial communities to achieve microbial adaptation against external stimuli *via* signaling molecules (autoinducers). The relative abundance of *cqsA*, which was related to CAI-1 autoinducer synthase (as the strongest autoinducer in quorum sensing) [31], was improved to 120% in low PHMG, while it was depressed to 59% with high dosage of PHMG (Fig. 4C). Moreover, the extracellular polymeric substance (EPS) synthesis and secretion played vital roles in protecting cells from external invasion and increasing their resistance and tolerance to environmental stresses [32]. In this work, when exposed to PHMG, the relative abundance of *rpfC* and *rpfG*, which were involved in EPS synthesis and biofilm formation, were enhanced by 1.4 and 2.1 times in low PHMG reactor, while they were decreased by 0.4 and 0.6 times with high dosage of PHMG reactor, respectively.

Obviously, the functional anaerobic species in WAS fermentation systems was stimulated with improved metabolic activity to adapt to the low exposure of PHMG, but they could not tolerate the high level of PHMG. In fact, the ATP level, which was an

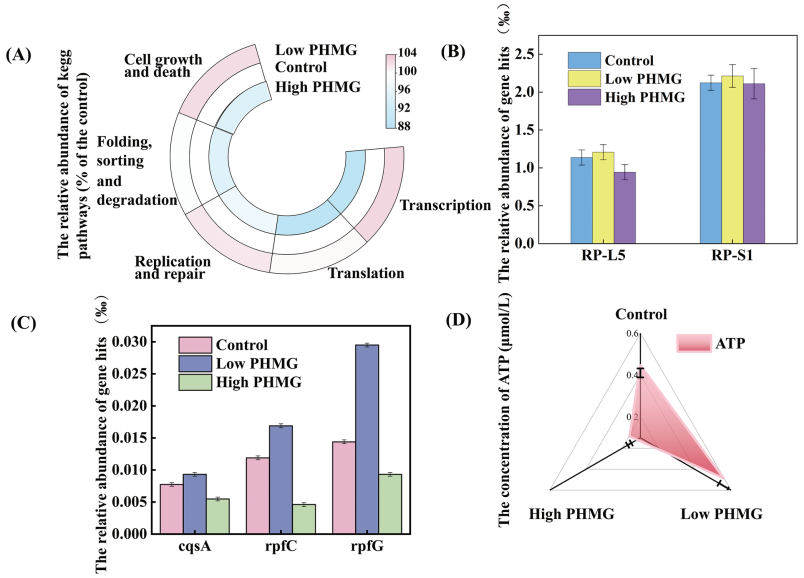

Fig. 4. (A) The relative abundances of critical functions based on KEGG classifications, (B) key genes responsible for translation, (C) key gene responsible for quorum sensing, and (D) ATP level release in different reactors.

irreplaceable bioenergy in living organisms and reflected the general microbial activity, was increased from 0.41 μmol/L in the control reactor to 0.56 μmol/L with low dosage of PHMG. But it was reduced to 0.16 μmol/L with the exposure to high contents of PHMG (Fig. 4D). The adaption of microorganisms to different levels of PHMG might account for the variations of microbial function traits, resulting in ultimately distinct VFAs production efficiency during WAS fermentation.

In summary, this work demonstrated the hormesis effects of PHMG on VFAs generation during WAS fermentation. The low presence of PHMG simultaneously enhanced the solubilization, hydrolysis, and acidification while inhibiting methanogenesis. Further analysis indicated the functional anaerobe and the corresponding vital genes (especially for protein metabolism) responsible for VFAs generation were improved in the low level of PHMG while reducing under high PHMG stress. The analysis of microbial adaptation proved that anaerobic species were able to respond and adapt to certain levels of stimuli driven by the PHMG. However, the excessive presence of PHMG posed evident toxicity to microorganisms and greatly reduced the metabolic activities, resulting in extremely low VFAs production efficiency.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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References

[1] S. Fang, W. Cao, Q. Shao, et al., J. Cleaner Prod. 349 (2022) 131456.
[2] L. Feng, W. Zhao, Y. Liu, et al., Chin. Chem. Lett. 34 (2023) 107439.
[3] Q. Fu, D. Wang, X. Li, et al., Renew. Sust. Energ. Rev. 135 (2021) 110283.
[4] J. Zhao, D. Wang, X. Li, et al., Water Res. 78 (2015) 111–120.
[5] M. Atasoy, O. Eyice, A. Schnürer, Z. Cetecioglu, Bioresour. Technol. 292 (2019) 121889.
[6] F. Xue, J.J.L.M. Cornelissen, Q. Yuan, S. Cao, Chin. Chem. Lett. 34 (2023) 107448.
[7] Q. Zhang, L. Wu, J. Huang, et al., Resour. Conserv. Recycl. 182 (2022) 106342.
[8] J. Luo, S. Fang, W. Huang, et al., Front. Environ. Sci. Eng. 16 (2021) 106.
[9] Y. Wang, M. Gu, D. Ge, et al., J. Environ. Manage. 315 (2022) 115146.
[10] J. Luo, F. Wang, X. Cheng, et al., J. Cleaner Prod. 315 (2021) 128145.
[11] S.P. Dharupaneedi, S.K. Nataraj, M. Nadagouda, et al., Sep. Purif. Technol. 210 (2019) 850–866.
[12] W.E. Federation, A. Association, Standard Methods For the Examination of Water and Wastewater, American Public Health Association (APHA), Washington DC, U. S. A., 2005.
[13] C. Uwineza, A. Mahboubi, A. Atmowidjojo, et al., Bioresour. Technol. 337 (2021) 125410.
[14] J. Luo, W. Huang, Y. Zhu, et al., Sci. Total Environ. 705 (2020) 135878.
[15] J. Luo, W. Huang, W. Guo, et al., Chem. Eng. J. 398 (2020) 125560.
[16] T.P. Moest, S. Méresse, Curr. Opin. Microbiol. 16 (2013) 38–44.
[17] C. Durmort, J.S. Brown, Chapter 10 - streptococcus pneumoniae lipoproteins and ABC transporters, in: J. Brown, S. Hammerschmidt, C. Orihuela (Eds.), Streptococcus Pneumoniae, Academic Press, Amsterdam, 2015, pp. 181–206.
[18] K. Liu, Y. Chen, N. Xiao, X. Zheng, M. Li, Environ. Sci. Technol. 49 (2015) 4929–4936.
[19] Y. Chen, X. Jiang, K. Xiao, et al., Water Res. 112 (2017) 261–268.
[20] E. Jankowska, J. Chwialkowska, M. Stodolny, P. Oleskowicz-Popiel, Chem. Eng. J. 326 (2017) 901–910.
[21] M.T. Wong, D. Zhang, J. Li, et al., Biotechnol. Biofuel. 6 (2013) 38.
[22] H.D. Ariesyady, T. Ito, S. Okabe, Water Res. 41 (2007) 1554–1568.
[23] J. Luo, Y. Li, W. Huang, et al., Chin. Chem. Lett. 34 (2023) 107661.
[24] K. Wen, A. Zhou, J. Zhang, et al., Sci. Rep. 7 (2017) 42887.
[25] W. Zhan, L. Li, Y. Tian, et al., Chem. Eng. J. 420 (2021) 129809.
[26] A. Sánchez, Resources, Environ. Dev. Sustain. 8 (2022) 100062.
[27] R.D.A. Cayetano, J. Park, G.B. Kim, J.H. Jung, S.H. Kim, Bioresour. Technol. 332 (2021) 125014.
[28] F. Wang, J. Luo, S. Fang, et al., J. Environ. Sci. 115 (2022) 253–264.
[29] X. Duan, Y. Chen, L. Feng, Q. Zhou, Water Res. 196 (2021) 117004.
[30] P. Skorski, P. Leroy, O. Fayet, M. Dreyfus, S.H.L. Denmat, J. Bacteriol. 188 (2006) 6277–6285.
[31] R. Kelly, M. Bolitho, D. Higgins, et al., Nat. Chem. Biol. 5 (2009) 891–895.
[32] F. Tao, Y.W. He, D.H. Wu, S. Swarup, L.H. Zhang, J. Bacteriol. 192 (2010) 1020–1029.