

Influence of particle size distribution on anaerobic degradation of phenol and analysis of methanogenic microbial community

Wang, Jing; Wu, Benteng; Sierra, Julian Muñoz; He, Chunhua; Hu, Zhenhu; Wang, Wei

DOI

[10.1007/s11356-020-07665-z](https://doi.org/10.1007/s11356-020-07665-z)

Publication date

2020

Document Version

Final published version

Published in

Environmental Science and Pollution Research

Citation (APA)

Wang, J., Wu, B., Sierra, J. M., He, C., Hu, Z., & Wang, W. (2020). Influence of particle size distribution on anaerobic degradation of phenol and analysis of methanogenic microbial community. *Environmental Science and Pollution Research*, 27(10), 10391-10403. <https://doi.org/10.1007/s11356-020-07665-z>

Important note

To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Copyright

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy

Please contact us and provide details if you believe this document breaches copyrights.
We will remove access to the work immediately and investigate your claim.



Influence of particle size distribution on anaerobic degradation of phenol and analysis of methanogenic microbial community

Jing Wang¹ · Benteng Wu¹ · Julian Muñoz Sierra^{2,3} · Chunhua He¹ · Zhenhu Hu¹ · Wei Wang¹

Received: 22 May 2019 / Accepted: 7 January 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Sludge morphology considerably affects the mechanism underlying microbial anaerobic degradation of phenol. Here, we assessed the phenol degradation rate, specific methanogenic activity, electron transport activity, coenzyme F₄₂₀ concentration, and microbial community structure of five phenol-degrading sludge of varying particle sizes (i.e., < 20, 20–50, 50–100, 100–200, and > 200 μm). The results indicated an increase in phenol degradation rate and microbial community structure that distinctly correlated with an increase in sludge particle size. Although the sludge with the smallest particle size (< 20 μm) showed the lowest phenol degradation rate (9.3 mg COD·gVSS⁻¹ day⁻¹), its methanogenic activity with propionic acid, butyric acid, and H₂/CO₂ as substrates was the best, and the concentration of coenzyme F₄₂₀ was the highest. The small particle size sludge did not contain abundant syntrophic bacteria or hydrogenotrophic methanogens, but contained abundant acetoclastic methanogens. Moreover, the floc sizes of the different sludge varied in important phenol-degrading bacteria and archaea, which may dominate the synergistic mechanism. This study provides a new perspective on the role of sludge floc size on the anaerobic digestion of phenol.

Keywords Anaerobic digestion · Phenol degradation · Particle size distribution · Phenol-degrading sludge · Methanogenic activity · Microbial community structure

Introduction

Phenolic compounds, naturally or artificially produced, have been identified as a major cause of performance failure in anaerobic digesters (Rosenkranz et al. 2013). The

concentration of phenol in the effluents of coal gasification, coking, petrochemical manufacturing, and paper industries was often as high as 10–17 × 10³ mg/L (Veeresh et al. 2005). Phenol has a toxic effect on all living organisms and leads to protein denaturation and coagulation (Hou et al. 2018; Shi et al. 2018). Therefore, the removal of phenol is essential for the protection of environment and human health. Currently, biodegradation of phenol is generally preferred to physico-chemical methods of removal because of lower treatment costs and a higher probability of complete mineralization. Phenol can be converted into methane under anaerobic conditions making anaerobic degradation of phenol one of the most attractive and cost-effective treatment methods (Ju et al. 2018; Ramakrishnan and Gupta 2006). For instance, the concentration of phenol in coal gasification wastewater generally ranges from 500 to 3000 mg/L, and the removal rate of phenol can reach 50–60% after anaerobic treatment (Wang et al. 2011b). Using upflow anaerobic sludge blanket (UASB) treatment, the concentration of phenol was reduced to 110–250 mg/L, with a hydraulic retention time (HRT) of 24 h (Wang et al. 2010, 2011a, b). Using anaerobic sequencing batch reactor (ASBR) and anaerobic expanded granule sludge

Responsible editor: Gerald Thouand

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11356-020-07665-z>) contains supplementary material, which is available to authorized users.

✉ Zhenhu Hu
zhhu@hfut.edu.cn

✉ Wei Wang
wang_wei@hfut.edu.cn; dwhit@126.com

¹ Department of Municipal Engineering, School of Civil Engineering, Hefei University of Technology, Hefei 230009, China

² Section Sanitary Engineering, Department of Water Management, Delft University of Technology, Stevinweg 1, 2628 CN Delft, The Netherlands

³ KWR Watercycle Research Institute, Groningehaven 7, 3430 BB Nieuwegein, The Netherlands

bed (EGSB) operations, with a HRT of 48 h, the phenol concentration reduced to 110 and 140–160 mg/L, respectively (Li et al. 2014; Zhao et al. 2013). However, high concentration of phenol frequently threatened the physical morphology and microbial community structure of phenol-degrading bacteria and even led to performance failure (Li et al. 2019).

In recent years, the granulation of sludge has successfully accelerated the start-up of anaerobic reactors treating phenolic wastewater. Anaerobic granular sludge has high mass transfer resistance, reducing the toxicity of phenol to microorganisms (Muñoz Sierra et al. 2017); however, some issues with anaerobic granular sludge need to be overcome, such as long formation periods when the proper seed is not available and strict operational parameter controls (Chen et al. 2018). Consequently, anaerobic membrane bioreactors were employed to treat phenol-containing wastewater (Muñoz Sierra et al. 2018; Wang et al. 2017b). It has previously been reported that the particle size of phenol-degrading sludge shows a descending trend in anaerobic membrane bioreactors with excellent performance (Muñoz Sierra et al. 2017). Li et al. (2018b) showed that with an increase in phenol concentration in the anaerobic biofilter reactor, the removal rate of phenol reached a maximum value, while the proportion of small particle size sludge gradually increased. It was also found that with a decrease in particle size in the UASB reactor, phenol utilization rate increased (Sierra et al. 2019). Interestingly, both the granular and small-flocculent sludge could achieve excellent removal of phenol under optimum operational conditions. Although the composition of the microbial communities was different under various phenolic wastewater treatment conditions, the phyla Chloroflexi, Thermotogae, Cloacimonetes, Firmicutes, Proteobacteria, Synergistetes, and Euryarchaeota were identified in every phenol-degrading sludge (Wang et al. 2017c). Compared to the change in the abundance of other bacteria, the abundance of the phylum Proteobacteria increased along with the size of sludge particles (Chen et al. 2018), while the abundance of the genus *Methanosaeta* (belonging to the phylum Euryarchaeota) increased when the particle size decreased (Sierra et al. 2019). The morphology of the sludge was clearly related to the metabolic characteristics and community structure of the microorganisms (Huang et al. 2018); however, to the best of our knowledge, the mechanism underlying phenol degradation in size-distributed phenol-degrading sludge has not been elucidated to date.

A summary of the information on anaerobic phenol-degrading sludge reported in the literature to date is presented in Table 1. The variation in sludge morphology was observed in many studies on anaerobic phenol-degrading reactors, but only a few of them examined the relationship between sludge particle size and phenol degradation. In this study, phenol degradation rate and methanogenic activity of size-distributed phenol-degrading sludge (i.e., < 20, 20–50, 50–

100, 100–200, and > 200 μm) were evaluated. Additionally, a comprehensive analysis of the microbial community structure of the size-distributed phenol-degrading sludge was performed.

Materials and methods

Inoculums and experimental design

The inoculum was obtained from a laboratory-scale UASB reactor treating synthetic phenolic wastewater. The sludge was then sieved in 20, 50, 100, and 200 μm mesh to divide the samples into five subsamples with different particle size ranges, namely, < 20 μm , 20–50 μm , 50–100 μm , 100–200 μm , and > 200 μm . The volatile suspended solids (VSS) of the five tested sized sludges were 0.20, 8.48, 15.96, 23.71, and 29.41 g/L, respectively. The total suspended solid (TSS) of each sludge was 3.01, 17.72, 26.47, 35.16, and 40.61 g/L, respectively. The experiment consisted of the following four parts: (a) to determine the phenol degradation rate of each sludge; (b) to identify the methanogenic activity of each sludge with different substrates, including acetate, propionate, butyric acid, and H_2/CO_2 ; (c) to determine the potential electron transfer in each sludge based on coenzyme F_{420} and electron transport system activity; (d) to gain insight into the microbial community structure of the different sized sludges.

Substrate utilization rate and specific methanogenic activity of sludge

To evaluate the activity of phenol degraders in each size-distributed sludge, the phenol utilization rate was assessed. Batch tests were carried out in 250-mL serum bottles (with a working volume of 100 mL) and bottles were inoculated with different sized phenol-degrading sludge, 20 mg/L of phenol and nutrient medium. The ratio of sludge mass (gVSS) to phenol COD (g) was 40:1 (Wang et al. 2017c). The volume of each sludge used per set of experiments was 50, 9.43, 5.01, 3.37, and 2.72 mL, respectively (considering the low VSS concentration of the smallest particle size sludge, it was added after concentration). The composition of the nutrient solution was as follows (in mg/L): $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 62.40; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 136.95; NH_4Cl , 680; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 36; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 32; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.8; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.2; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.8; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.012; ZnCl_2 , 0.02; Na_2WO_4 , 0.032; HBO_3 , 0.02; $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$, 0.04; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.036; $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.02. The bottles were purged with N_2 for 1–2 min to create anaerobic conditions, then sealed with rubber stoppers, and cultivated at 35 °C in a shaker at 140 rpm. Liquid samples were collected every 4 h with a syringe and filtered through a 0.45-mm filter prior to liquid chromatography (LC) analysis. The phenol concentration of the liquid

Table 1 Information of anaerobic phenol-degrading sludge reported in the literatures

Sludge type	Size	Main substrates	Reactor type	Phenol concentration	Tem	pH	HRT	Reference
Granule	1.0–3.0 mm	Phenol	UASB	105–1260 mg/L	37 °C	7.0	24–12 h	Tay et al. (2001)
Granule	1.5–4.0 mm	Phenol, glucose	UASB	105–1260 mg/L	37 °C	7.0	24–12 h	Tay et al. (2001)
Floc	*56 µm (18 Na ⁺ /L) *41 µm (24 Na ⁺ /L) *16 µm (26 Na ⁺ /L)	Phenol, Na ⁺	AnMBR	0.5–3.0 g/L	35 ± 0.8 °C	8.0 ± 2.0	7 days	Sierra et al. (2019)
Granule, floc	*146 µm (18 Na ⁺ /L) *91 µm (24 Na ⁺ /L) *41 µm (26 Na ⁺ /L)	Phenol, Na ⁺	UASB	0.5–3.0 g/L	35 ± 0.8 °C	8.0 ± 2.0	7 days	Sierra et al. (2019)
Granule	*110.3–136.7 µm	Phenol, acetate	ABCMBR	500 mg/L	35 ± 1 °C	–	48 h	Wang et al. (2017b)
Granule	0.5–1.5 mm	Phenol, p-cresol	UASB	600–800 mg/L	37 °C	7.5–8.1	12–2 h	Fang and Zhou (1999)
Granule	0.5–1.5 mm	Phenol, p-cresol	UASB	680–2500 mg/L	37 °C	7.5–8.2	24 h	Fang and Zhou (1999)
Granule	1.0–2.0 mm	Phenol	UASB	420–1290 mg/L	37 °C	6.8–7.5	8–12 h	Fang et al. (1996)
Granule	0.66–0.75 mm	Phenol	UASB	150–500 mg/L	37 °C	6.5–7.5	–	Fukuzaki et al. (1991)

*The median particle size median size (D50). The symbol ‘–’ represents that this information was not mentioned in the literature

UASB up-flow anaerobic sludge blanket reactor, AnMBR anaerobic membrane bioreactor, ABCMBR anaerobic baffled ceramic membrane bioreactor

samples was determined using high-performance liquid chromatography (HPLC, 1260 Infinity, Agilent Inc., USA) with a C18 column (4.6 × 250 mm). The SUR of the sludge was determined by calculating the slope of phenol reduction over time as mgCOD·gVSS⁻¹ day⁻¹. The specific methanogenic activity (SMA) of each sludge was determined using the following four substrates: acetate, propionate, butyric acid, and H₂/CO₂ (80/20 v/v), and samples were collected every 2 h. The procedure was repeated for phenol utilization rate batch tests. Methane content of biogas was analyzed using a gas chromatograph (GC, SP-6890, Shandong Ruihong Ltd., China). The SMA of each sludge was determined by calculating the slope of methane production and expressed as gCOD-CH₄·gVSS⁻¹ day⁻¹. The tests were carried out in triplicate and the standard deviation of three sets of parallel sample data was set as error bars. Data on the SMA of propionic and butyric acid in the seed sludge are calculated based on mass distribution. Data analysis was performed using Origin Pro 8.5.

Coenzyme F₄₂₀ and electron transport system activity tests

Sludge samples were collected after centrifugation at 16,000×g for 30 min at 4 °C and being washed twice with saline solution (0.9% NaCl) at 4 °C. Samples were then immersed in a saline solution for 30 min and centrifuged to remove the supernatant. Distilled water was added to each centrifuge tube to a volume of 30 mL and heated in a water bath at 95–100 °C for 30 min. Samples were stirred every 10 min using a glass rod. Cooled samples were then centrifuged at 10,000×g for 15 min and 10 mL supernatant was added to 20 mL isopropyl alcohol, following which they were left to precipitate in the dark for 2 h. Samples were centrifuged again (10,000×g, 10 min) to obtain a supernatant, which was

divided in half. One portion was adjusted to pH 1.0 with 6 mol/L HCl and was used as a blank group, while the second portion was adjusted to pH 13.5 with 6 mol/L NaOH as the test group. A spectrophotometer, under 420 nm extraction wavelength, was used to analyze the coenzyme F₄₂₀ assay (Heine-Dobbernack et al. 1988).

Electron transport system activity was determined using 2-para (iodo-phenyl)-3(nitrophenyl)-5(phenyl) tetrazolium chloride (INT) (Wang et al. 2016). For each size-distributed sludge, 0.3-mL homogenized sludge sample was added to a 10-mL centrifuge tube and treated as per the protocol devised by Wang et al. (2016). Data on F₄₂₀ and ETS of seed sludge are calculated based on mass distribution. The electron transport activity was calculated as follows:

$$U = 210.53 \cdot \frac{D_{485}}{W} \tag{1}$$

where *U* is the electron transfer system activity, *D*₄₈₅ is the absorbance at 485 nm, and *W* is the dry weight of the sludge.

Analysis of microbial community structure

Samples were collected from each size-distributed sludge to analyze the bacterial and archaeal community structure. Samples were analyzed by 16S rRNA gene high-throughput sequencing. The 16s rRNA gene was amplified using the V3-V4 region universal primers 341F (5-CCTACGGG NGGCWGCAG-3) and 805R (5-GACTACHVGGGTATCT AATCC-3). Samples were subjected to high throughput sequencing using the Illumina Miseq platform (Illumina, Inc., San Diego, CA, USA). The extraction and sequencing were then performed using Shanghai Sangon Bioengineering Technology Service Co., Ltd. pipeline. The read numbers of

the five samples were 41685, 47076, 44998, 51827, and 46198, respectively, with the average read length being 416.20, 414.14, 417.34, 422.74, and 422.05, respectively. Coverage of all samples was 0.97. The measurement could only be made after the quality met the requirements. Based on weighted UniFrac, principal coordinate analysis (PCoA) was used to compare the similarity of different samples.

Results and discussion

Substrate utilization rates of size-distributed phenol-degrading sludge

The substrate utilization rates (SUR) for each size-distributed sludge are shown in Fig. 1 and Fig. S1. The results indicated that the smallest particle size sludge (< 20 μm) had the slowest phenol degradation rate (9.30 $\text{mgCOD}\cdot\text{gVSS}^{-1}\text{ day}^{-1}$), while the largest particle size sludge (> 200 μm) had the highest phenol degradation rate (21.30 $\text{mgCOD}\cdot\text{gVSS}^{-1}\text{ day}^{-1}$). The SUR of the sludges of other particle sizes, namely 20–50 μm , 50–100 μm , and 100–200 μm , were 11.30, 17.30, and 17.80 $\text{mgCOD}\cdot\text{gVSS}^{-1}\text{ day}^{-1}$, respectively. The SUR of the seed sludge was 28.60 $\text{mgCOD}\cdot\text{gVSS}^{-1}\text{ day}^{-1}$. It has previously been shown that bigger particle size sludges have higher organic or phenolic degradation rates (Chen et al. 2018; Tay et al. 2001), so it was expected that as the sludge particle size increased, the phenol degradation rate of the sludge gradually increased as well. Floc and granule sludge differed in structure and physiology, thus differed in their microbial interaction (Wu et al. 2016). Similarly, Luo et al. (2017) found that ammonia-oxidizing sludge with different particle sizes had different microbial community structures and that the inner and outer layers of granular sludge could cooperate to accomplish better autotrophic nitrogen removal.

Different particle size anaerobic sludge differed in microbial structure and characteristics. It has been shown previously that small and medium-size anaerobic granular sludge had similar pore structure but were significantly different from large granule sludge (Wu et al. 2016). Anaerobic degradation of phenol requires the cooperation of functional microorganisms, so the compactness of microbes may further accelerate substrate transfer. Sludge particle size and compactness play an important role in anaerobic reactors, and it has been shown that larger sized sludge flocs have a higher mass transfer rate than smaller particle sludge (Afridi et al. 2018). Sludge cultured with phenol was shown to have a variety of dense bacteria throughout the sludge fractions, and the VFAs formed in the sludge could easily be converted into methane by the surrounded methanogens (Chang et al. 1995; Chou and Huang 2005). The internal arrangement of large particle size sludge may be beneficial for the conversion of substrates and intermediates and could create the best metabolic conditions

for all its constituents (Subramanyam and Mishra 2013). The > 200 μm sludge contributed about fivefold more to the SUR of seed sludge than the < 20 μm sludge did; therefore, the structure of larger particle size phenol-degrading sludge accelerated substrate uptake and resulted in a higher phenol degradation rate, as shown in Fig. 1.

Specific methanogenic activity of size-distributed phenol-degrading sludge

The SMA of each phenol-degrading sludge with acetate, propionate, butyrate, and H_2/CO_2 as a substrate is shown in Fig. 2. The SMA-acetate value of sludge was enhanced with the increase of sludge particle size, with the highest SMA-acetate value of $2.06 \pm 0.10\text{ gCOD}\cdot\text{CH}_4\cdot\text{gVSS}^{-1}\text{ day}^{-1}$ in the largest particle size sludge (> 200 μm) (Fig. 2a). Interestingly, the highest SMA-propionate, SMA-butyrate, and SMA- H_2/CO_2 values, 0.72, 0.91, and 1.71 $\text{gCOD}\cdot\text{CH}_4\cdot\text{gVSS}^{-1}\text{ day}^{-1}$, respectively, were obtained in the smallest particle size sludge (< 20 μm). The SMA value of the seed sludge with acetate, propionic acid, butyric acid, and H_2/CO_2 was 1.72, 0.07, 0.51, and 0.38 $\text{gCOD}\cdot\text{CH}_4\cdot\text{gVSS}^{-1}\text{ d}^{-1}$, respectively. The > 200 μm sludge contributed about fourfold more to the SMA-acetate value of seed sludge than the < 20 μm sludge, while the < 20 μm sludge contributed about 18 times more to the SMA-propionic value of the seed sludge than the > 200 μm sludge. The contribution of the < 20 μm and > 200 μm sludge to the seed sludge SMA-butyric values was similar.

The reaction to convert butyric acid and propionic acid to acetate is energy consuming and cannot be carried out under high hydrogen partial pressure conditions (Viggi et al. 2014; Zhang et al. 2018a). The conversion rate of hydrogen to methane in the smallest particle size sludge (< 20 μm) was,

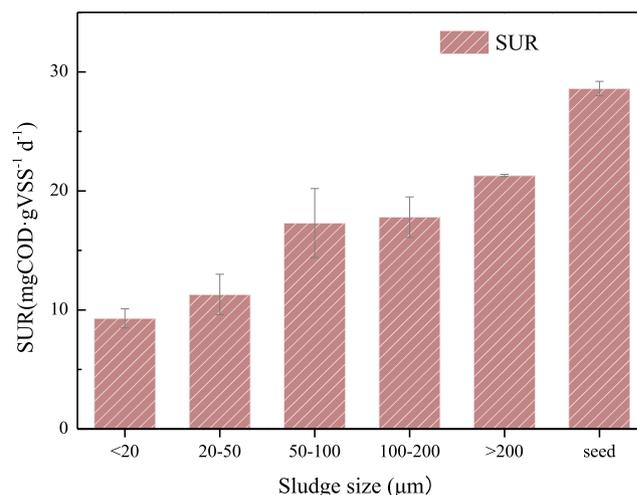


Fig. 1 Substrate utilization rate (SUR) of size-distributed phenol-degrading sludge ('seed' represents the seed sludge without any size segregation; '< 20, 20–50, 50–100, 100–200, and > 200' represent the sludge samples with particle size ranges of < 20 μm , 20–50 μm , 50–100 μm , 100–200 μm , and > 200 μm , respectively)

however, very high, with the $< 20 \mu\text{m}$ sludge contributing about fivefold more to the SMA- H_2/CO_2 value of seed sludge than the $> 200 \mu\text{m}$ sludge, indicating high hydrogen uptake and electronic uptake ability which indirectly promoted the degradation rate of propionic and butyric acid. The SMA- H_2/CO_2 value of large particle size sludge was relatively low. H_2/CO_2 was easily utilized by the methanogens in the smallest particle size sludge, suggesting the likelihood of multiple degradation pathways for accelerating the utilization rate of propionic, butyric acid, and H_2/CO_2 , such as directly utilizing hydrogen (Wang et al. 2017c), transforming hydrogen into acetic acid by homoacetogens (Wang et al. 2013), or by syntrophic interactions (Zhao et al. 2016).

Potential electron transfer and coenzyme F_{420} concentration of size-distributed phenol-degrading sludge

The electron transfer system activity and F_{420} concentration of size-distributed phenol-degrading sludge are presented in Fig. 3. Coenzyme F_{420} presented a useful method to assess potential methanogenic capacity, especially the H_2 -utilizing capacity of the sludge (Yin et al. 2018). Although the F_{420} value of the seed sludge was only $0.28 \mu\text{g}\cdot\text{gVSS}^{-1}$, the concentration of F_{420} in the smallest particle size sludge ($< 20 \mu\text{m}$) reached $2.51 \mu\text{g}\cdot\text{gVSS}^{-1}$, which was much higher than that of the large particle size sludge. The 20–50 μm sludge had the highest electron transfer system activity value ($12.17 \mu\text{g mg}^{-1} \text{day}^{-1}$). In previous studies, coenzyme F_{420} had only been found under methanogenic condition and was used to reflect the activity of methane-producing microorganisms (Dolfing and Mulder 1985). As an electron carrier, it played a key metabolic role in both anabolic and catabolic redox reactions in methanogenic bacteria (Dolfing and Mulder 1985). The F_{420} concentration tended to increase with the sludge particle size, except at $< 20 \mu\text{m}$, which was consistent with the tendency of SMA-acetate. The F_{420} concentration in the smallest particle size sludge ($< 20 \mu\text{m}$) was comparable to SMA- H_2/CO_2 , implying that coenzyme F_{420} was closely correlated to hydrogenotrophic activity. Liu et al. (2017) also indicated that coenzyme F_{420} played an important role in the hydrogenotrophic pathway and was required for the final reaction steps of the methanogenic pathway. In addition, the high F_{420} concentration in the smallest particle size sludge correlated strongly the high utilization rate of hydrogen and degradation rate of propionic and butyrate acid.

The highest ETS value ($12.17 \pm 1.00 \mu\text{g mg}^{-1} \text{day}^{-1}$) was observed in the 50–100 μm particle size sludge (Fig. 3b), while the lowest ETS value ($2.99 \pm 0.17 \mu\text{g mg}^{-1} \text{day}^{-1}$) was found in the smallest particle size sludge ($< 20 \mu\text{m}$). ETS activity can be used to estimate the biological respiratory activity and bioactivity of sludge (Yin et al. 2018; Zhang et al. 2018b). Syntrophic bacteria and methanogens can achieve

methanogenesis of phenol by extracellular electron transfer (Yan et al. 2018); however, the electron transport system activity of the small particle size sludge was weak.

Microbial community structure of size-distributed phenol-degrading sludge

Bacteria

The results of principal coordinate analysis (PCoA) based on weighted unifrac distance of bacterial communities in each of the five samples are shown in Fig. 4a. The smallest particle size sludge ($< 20 \mu\text{m}$) located far away from the other samples, indicating a significant difference in bacterial community structure compared to the other particle size sludges. Similar bacterial community structure was observed between the 100–200 μm and $> 200 \mu\text{m}$ particle size sludge, indicated by the short unifrac distance between the two. Based on the PCoA results, we further identified the microbial community structure at the phylum and genus levels, which are shown in Fig. 4b and c.

At the phylum level (Fig. 4b), Bacteroidetes (24.91%), Proteobacteria (17.32%), Thermotogae (10.26%), Synergistetes (11.75%), Firmicutes (11%), Euryarchaeaota (9.1%), and Chloroflexi (8.44%) were dominant in the smallest particle size sludge. The relative abundance of the phyla Cloacimonetes (3.8–15.37%) and Proteobacteria (19–34.29%) in larger particle size sludge was greater than that of the smallest particle size sludge. The relative abundance of the phyla Bacteroidetes (24.91–5.47%) and Euryarchaeaota (9.1–0.27%) decreased with an increase in sludge particle size.

At the genus level (Fig. 4c), the relative abundances of the functional syntrophic phenol degraders *Syntrophus* (2.27% to 29.12%), *Candidatus Cloacamonas* (1.49–15.37%), and *Pelotomaculum* (1.49% to 15.37%) increased along with particle size. The relative abundance of the syntrophic bacteria *Mesotoga*, *Thermovirga*, and *Levilinea*, in all five particle sizes of sludge, was relatively high with no significant difference between particle sizes. It has previously been reported that *Thermovirga* (belonging to the phylum Synergistetes) has strong hydrolytic ability (Wang et al. 2017a). The role of *Mesotoga* in anaerobic digestion has mainly been reported as a lactic acid utilizer (Goux et al. 2015), but the exact role of *Mesotoga* in phenol degradation requires further exploration. *Levilinea* (belonging to the phylum Chloroflexi) has often been found in phenolic treatment reactors. It is not only an important hydrolytic fermentative bacterium (Antwi et al. 2017), but it is also a primary acidogenic/acetogenic bacteria in anaerobic digesters (Zhang et al. 2017). The microbial phyla Bacteroidetes, Firmicutes, Proteobacteria, and Chloroflexi have widely been reported in the anaerobic treatment of phenolic wastewater (Li et al. 2016; Na et al. 2016). The phylum Proteobacteria plays an important role in the key anaerobic

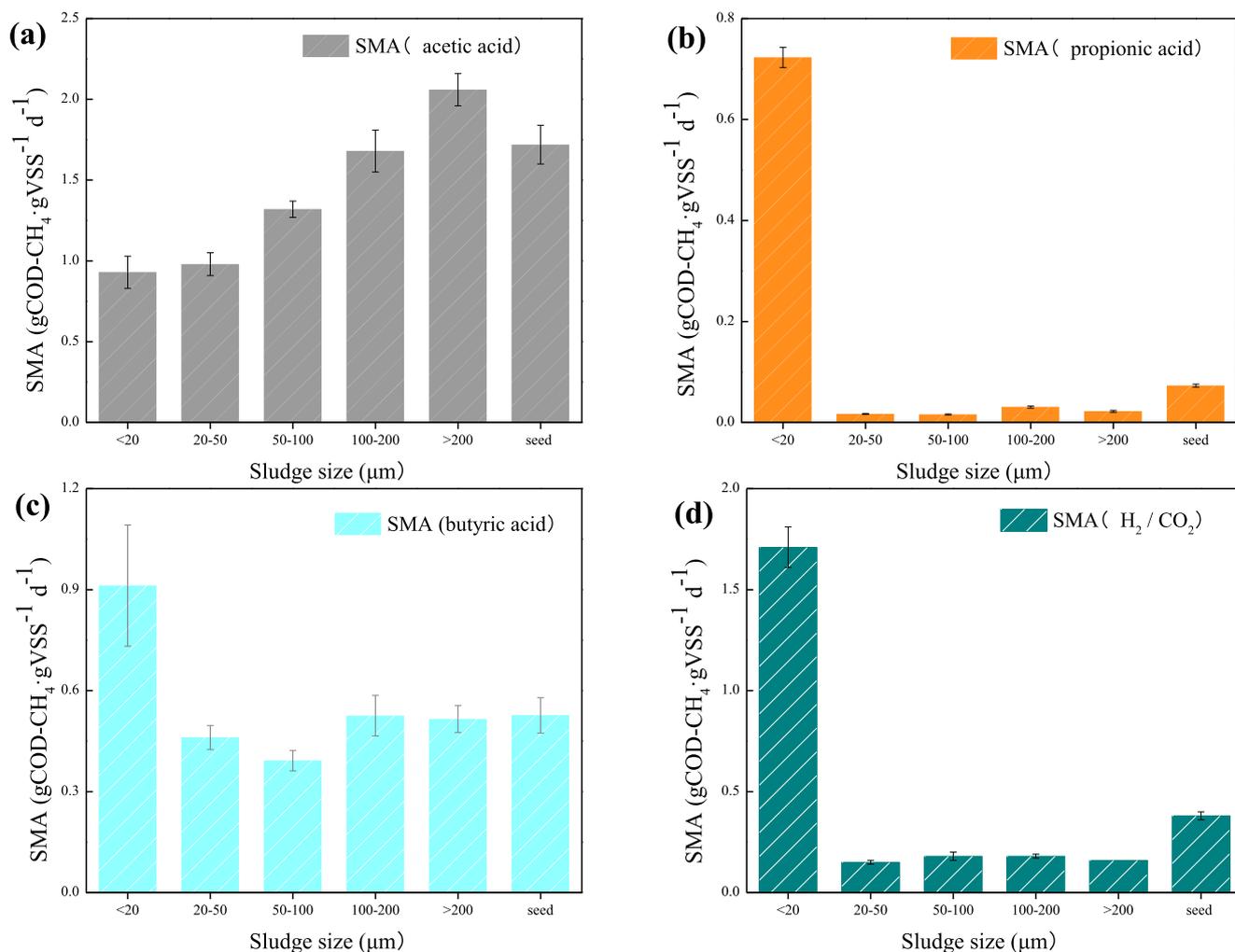


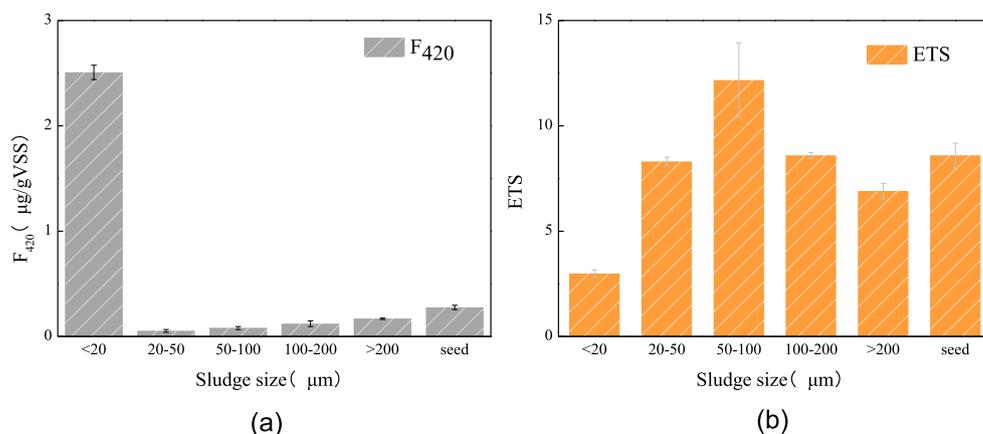
Fig. 2 Specific methanogenic activity (SMA) of size-distributed phenol-degrading sludge. **a–d** SMA-acetic acid, propionic acid, butyric acid, and H₂/CO₂, respectively. ‘seed’ represents the seed sludge without any size

segregation; ‘< 20, 20–50, 50–100, 100–200, and >200’ represent the sludge samples with particle size ranges of < 20 μm, 20–50 μm, 50–100 μm, 100–200 μm, and > 200 μm, respectively)

digestion steps of hydrolysis and acetogenesis (Qian et al. 2019; Wu et al. 2019). It has been reported that *Syntrophus* (belonging to the phylum Proteobacteria) intracellularly degrades benzoate into acetate and shows higher substrate

affinity to benzoate or higher growth rate (Chen et al. 2008). Since the conversion of phenol to benzoate is a rate-limiting step, the rapid consumption of benzoate promoted the forward reaction of phenol conversion. The sludge with the smaller

Fig. 3 F₄₂₀ (a) and ETS activity (b) of size-distributed phenol-degrading sludge. F₄₂₀ represents the concentration of coenzyme F₄₂₀; ETS represents the electron transfer system. ‘Seed’ represents the seed sludge without any size segregation; ‘< 20, 20–50, 50–100, 100–200, and > 200’ represent the sludge samples with particle size ranges of < 20 μm, 20–50 μm, 50–100 μm, 100–200 μm, and > 200 μm, respectively)



particle sizes of < 20 μm and 20–50 μm had a relatively low abundance of *Syntrophus*, indicating that the larger particle size sludges more than likely had the ability to convert phenol to benzoate. It has been shown that *Syntrophus* establishes syntrophic interactions with hydrogenotrophic methanogens through the H_2 -producing fermentation of various organic matter (McInerney et al. 2007). *Candidatus Cloacamonas*, belonging to the phylum Cloacimonetes, plays a putative role in the metabolism of fatty acids and amino acids (Ju and Zhang 2014; Svensson et al. 2018). Similar to *Syntrophus*, *Candidatus Cloacamonas* is also an important syntrophic bacterium, explaining why the larger particle size sludge had the better phenol degradation rate. *Pelotomaculum*, belonging to the phylum Firmicutes, has been often detected in reactors treating wastewater containing aromatics (Nobu et al. 2017). Species belonging to the phylum Firmicutes are acidogens or hydrolytic bacteria, which are conducive to the conversion of phenol to benzoate. Previously, a high phenol conversion rate has been shown to correlate with a relatively high abundance of *Pelotomaculum* (Muñoz Sierra et al. 2018). Chen et al. (2008) reported that *Desulfotomaculum subcluster Ih* contained *Pelotomaculum* spp., which could convert phenol to benzoate at 30–37 °C under anaerobic conditions. Our experimental data showed the largest particle size sludge exhibited the highest phenol degradation rate, which correlated to the highest relative abundance of *Pelotomaculum*. Furthermore, Na et al. (2016) stated that phenol was degraded by *Syntrophus* and *Pelotomaculum*, both of which were dominant in the largest particle size sludge and the high phenol degradation rate and SMA-acetate values could be attributed to their presence.

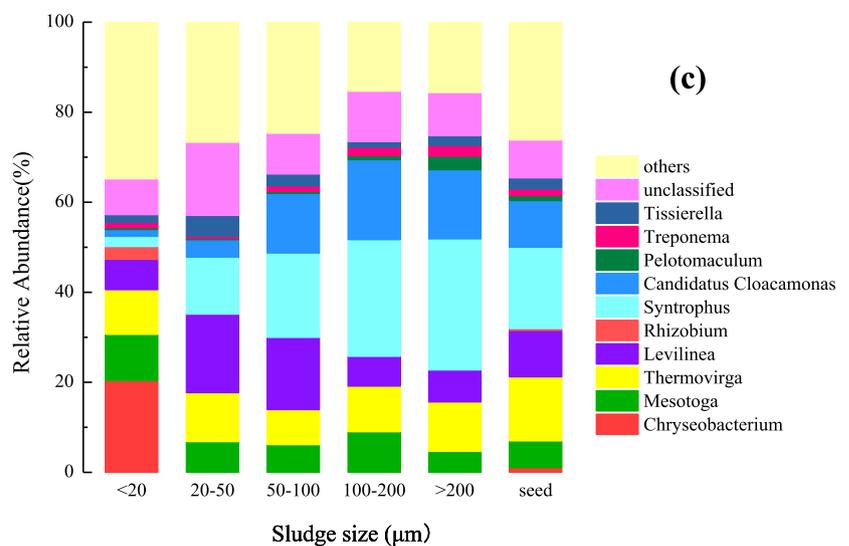
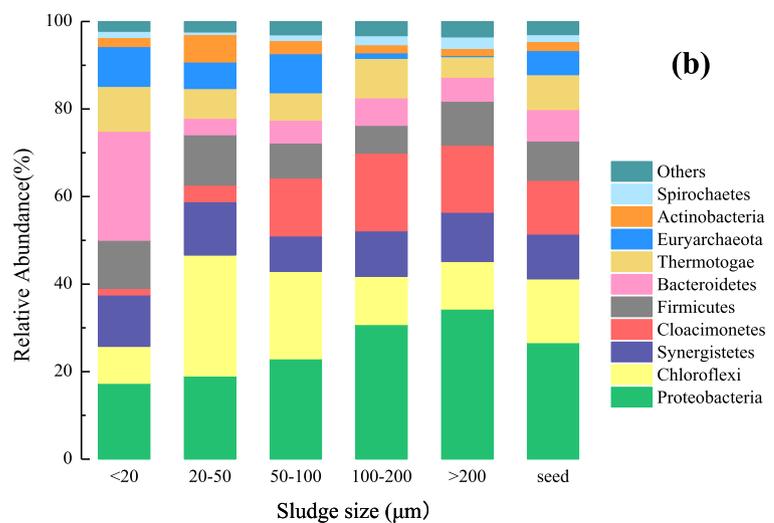
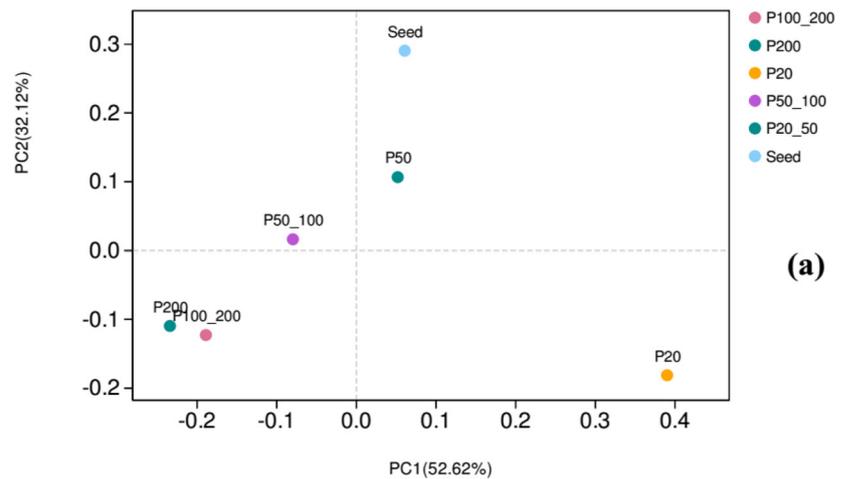
Interestingly, only the smallest particle size sludge contained the bacteria *Rhizobium*, some strains of which are known to efficiently recycle H_2 as an electron donor (Bretschger et al. 2015; Nguyen et al. 2017). Wei et al. (2008) and Gomez-Acata et al. (2017) reported that *Rhizobium* could utilize phenol as a carbon source. Lai et al. (2016) found *Rhizobium* to be the predominant genus in H_2/CO_2 -utilizing experiments. Likewise, Leandro et al. (2018) found elevated *Rhizobium* in H_2/CO_2 -fed cultures. It is, therefore, obvious that only the smallest particle size sludge, with its relatively high abundance of *Rhizobium*, showed a high H_2/CO_2 -utilizing rate, but the exact pathway through which *Rhizobium* utilizes H_2 remains unclear and requires further investigations. As the degradation of butyric and propionic acids is thermodynamically favorable under low partial hydrogen pressure (Xu et al. 2016), the smallest particle size sludge might be efficient for utilizing butyric and propionic acid, as indicated earlier. The genera *Chryseobacterium* (20.38%) and *Rhizobium* (2.85%) were only found in the smallest particle size sludge (< 20 μm). The genus *Chryseobacterium* has shown tolerance to a toxic environment (Loveland-

Curtze et al. 2010); thus, the smallest particle size sludge may play an important role in resisting harsh conditions.

Archaea

The principal coordinate analysis (PCoA) based on weighted unifracs distance of archaea communities in the five size samples is shown in Fig. 5a. Results show that the 50–100 μm , 100–200 μm , and > 200 μm particle size sludge have similar community structures. The seed sludge showed less similarity in community structure compared to the other samples. Analysis of the archaeal microbial community structure at the phylum and genus levels, based on the PCoA results, was conducted and is shown in Fig. 5b and c. The main archaea from the five samples were found to consist of four genera, of which three were hydrogenotrophic methanogens (*Methanofollis*, *Methanolinea*, and *Methanosphaera*) and one was an acetoclastic methanogen (*Methanosaeta*). Most archaea in the smallest particle size sludge (< 20 μm) were principally acetoclastic methanogens with *Methanosaeta* (belonging to the phylum Euryarchaeota) accounting for 92.6% of the relative abundance. With an increase in sludge particle size, the relative abundance of acetoclastic methanogens gradually decreased, and the types of methanogens tended to vary. *Methanosaeta* plays a significant role in granule formation by forming a skeleton within the granule to which other bacteria are able to attach (Sutton et al. 2013; Thauer et al. 2008). Previous studies have shown that *Methanosaeta* conducts direct interspecies electron transfer with other bacterial species, accelerating the symbiotic degradation of propionate and butyrate (Holmes et al. 2017; Zhao et al. 2016). *Methanosaeta* has been noted for its electron consumption capacity as an acetoclastic methanogen, especially the consumption of electrons released from the oxidation of butyrate or propionate to acetate (Wang and Li 2016), which could explain why the smallest particle size sludge had the highest SMA- butyrate and propionate values. In previously described anaerobic digesters, members of the phyla Synergistetes (represented by *Syntrophus*) and Firmicutes (represented by *Pelotomaculum*) were able to provide H_2 and short-chain acids through the degradation of organic acids, which helped to establish syntrophic relationships with hydrogenotrophic and acetoclastic methanogens (especially *Methanosaeta*) (Riviere et al. 2009; Zhang et al. 2005). As mentioned in section “Bacteria” of this paper, *Candidatus Cloacimonas* could establish syntrophic relationships with hydrogenotrophic methanogens. According to Figs. 4b and 5b, the smallest particle size sludge showed less relative abundance of these syntrophic bacteria. Although the smallest particle size sludge had high H_2 -utilizing potential, according to the F_{420} and SMA- H_2 values, it lacked an

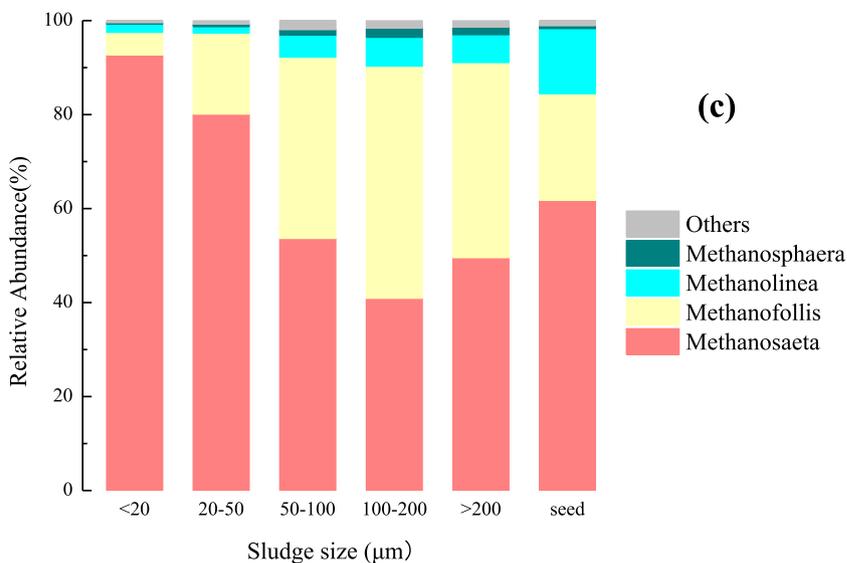
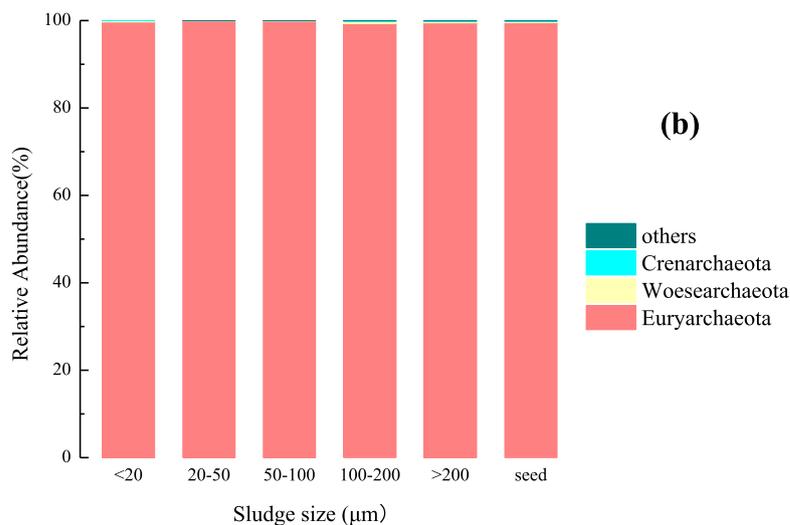
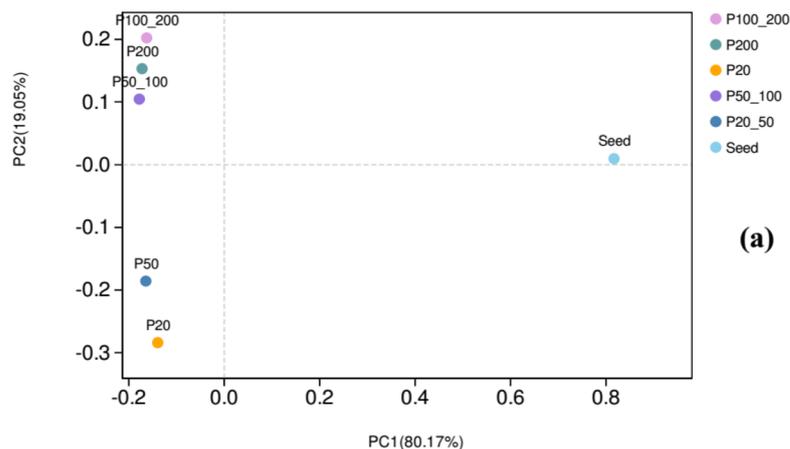
Fig. 4 **a** PCoA based on weighted unifrac distance of bacterial communities in size-distributed phenol-degrading sludge; relative abundances of the bacteria in the **b** phylum level, **c** genus level (“Other” represents all classified taxa that were < 1% in all samples). PCoA represents principal coordinates analysis. The symbols 20, 50, 50_100, 100_200, 200 and seed in **a–c** represent the sludge samples with particle size ranges of < 20 μm, 20–50 μm, 50–100 μm, 100–200 μm, > 200 μm and the seed sludge without any size segregation, respectively



effective syntrophic relationship. The smallest particle size sludge contained only a low abundance of hydrogenotrophic methanogens and abundant acetoclastic

methanogens; however, this sludge type exhibited a strong ability to convert H_2/CO_2 to methane. Combined with the *Rhizobium* distribution, we speculate that the smallest

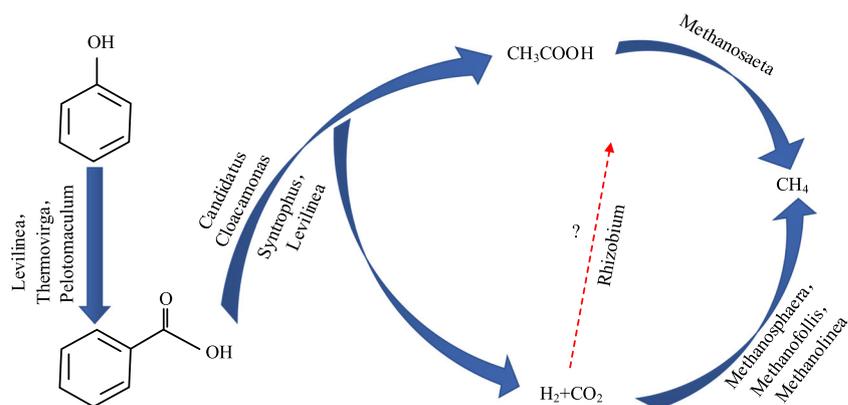
Fig. 5 **a** PCoA based on weighted unifracs distance of archaea communities in size-distributed phenol-degrading sludge; Relative abundances of the archaea in the **b** phylum level, **c** genus level (“Other” represents all classified taxa that were < 1% in all samples. PCoA represents principal coordinate analysis. The symbols 20, 50, 50_100, 100_200, 200, and seed in **a–c** represent the sludge samples with particle size ranges of < 20 μm, 20–50 μm, 50–100 μm, 100–200 μm, > 200 μm and the seed sludge without any size segregation, respectively)



particle size sludge can convert hydrogen to acetic acid through hydrogen utilizers such as *Rhizobium* bacteria, and then convert the acetic acid to methane through

acetoclastic methanogens. *Methanosaeta*, which belongs to the order Methanosarcinales (Veeresh et al. 2005), was the predominant acetoclastic methanogen among all

Fig. 6 Phenol degradation pathway with related bacteria and archaea



samples. As previously discussed, *Methanosaeeta* sp. highly express genes that encode enzymes involved in the reduction of acetate to methane (Town et al. 2014), cooperating with hydrogenotrophic methanogens. In our study, the abundance of *Methanofollis* increased with an increase in sludge particle size. Satoru et al. (2011) stated that the genus *Methanofollis* is always found in acetate-rich wastewaters. The role of *Methanolinea* in phenol degradation is not clear, except as H₂ scavengers (Li et al. 2018a). *Methanofollis* and *Methanolinea* belong to the order Methanomicrobiales, which is associated with fatty acid syntrophic degradation in large particle size sludge.

The type of methanogen in the smallest particle size sludge was almost exclusively acetoclastic, so its syntrophic degradation ability was not better than that of the large particle size sludge. Madigou et al. (2016) indicated that hydrogenotrophic methanogenesis was preferred at high concentrations of phenol. The large particle size sludge had reasonable proportions of acetoclastic and hydrogenotrophic methanogens, leading to a high phenol degradation rate with excellent syntrophic association.

Based on the bacteria and archaea community analysis, we propose a phenol degradation network as shown in

Fig. 6, indicating the suggested relevant players in each step. The relative abundance of each of those bacteria and archaea are reflected in Table 2. Figure 6 highlights some important bacteria and archaea involved in anaerobic phenol degradation, especially syntrophic bacteria and methanogens. All bacteria and archaea in Table 2 have been discussed in detail in the previous sections. In general, compared to large granular sludge, especially the largest particle size sludge (> 200 μm), the smallest particle size sludge showed a poor variety of syntrophic bacteria and lacked strong syntrophic cooperation with methanogens.

Conclusion

Generally, in the sludge flocs, the smallest size part showed a higher rate in utilizing hydrogen and propionic acid, compared to the larger size part, while the larger particle part exhibited a higher utilization of acetate and phenol degradation. The larger particle size sludge also had abundant syntrophic bacteria and the dominant genera

Table 2 The related bacteria and archaea and their relative abundance

Type	Name	Relative abundance (%)					Seed
		< 20 μm	20–50 μm	50–100 μm	100–200 μm	> 200 μm	
Bacteria	<i>Levilinea</i>	6.75	17.44	16.06	6.63	7.1	10.31
	<i>Thermovirga</i>	9.91	10.86	7.76	10.11	10.92	14.21
	<i>Pelotomaculum</i>	0.4	0.28	0.47	0.9	3.01	1.13
	<i>Syntrophus</i>	2.27	12.7	18.72	25.93	29.12	18.13
	<i>Candidatus Cloacamonas</i>	1.49	3.8	13.25	17.76	15.37	10.31
	<i>Rhizobium</i>	2.85	0.03	0.00	0.00	0.00	0.42
	Archaea	<i>Methanosaeeta</i>	92.65	80.09	53.64	40.89	49.52
<i>Methanofollis</i>		4.84	17.26	38.54	49.4	41.53	22.72
<i>Methanolinea</i>		1.79	1.36	4.71	6.13	5.94	13.82
<i>Methanosphaera</i>		0.26	0.52	1.17	1.99	1.66	0.61

in the smallest particle size sludge were mostly related to hydrogen utilization and environment adaption. Phenol degradation is clearly a process of syntrophic cooperation, substantiated by the sludge flocs showing the better phenol utilization ability than any of the different sized sludge. Whatever the size of sludge flocs, the smallest size sludge is a beneficial supplement to establish a strong syntrophic cooperation in the sludge flocs. This study has been instrumental in elucidating the underlying microbial synergistic mechanisms in the sludge flocs and their alterations related to the changes in sludge morphology.

Funding information This study was funded by the National Natural Science Foundation of China (51878232) and CAS Key Laboratory of Urban Pollutant Conversion, University of Science and Technology of China (KF201702).

References

- Afridi ZUR, Wu J, Li ZH, Akand R, Cao ZP, Poncin S, Li HZ (2018) Novel insight of spatial mass transfer conditions of upflow anaerobic reactor. *J Clean Prod* 204:390–398
- Antwi P, Li J, Boadi PO, Meng J, Shi E, Xue C, Zhang Y, Ayivi F (2017) Functional bacterial and archaeal diversity revealed by 16S rRNA gene pyrosequencing during potato starch processing wastewater treatment in an UASB. *Bioresour Technol* 235:348–357
- Bretschger O, Carpenter K, Phan T, Suzuki S, Si I, Grossi-Soyster E, Flynn M, Hogan J (2015) Functional and taxonomic dynamics of an electricity-consuming methane-producing microbial community. *Bioresour Technol* 195:254–264
- Chang Y-J, Nishio N, Nagai S (1995) Characteristics of granular methanogenic sludge grown on phenol synthetic medium and methanogenic fermentation of phenolic wastewater in a UASB reactor. *J Ferment Bioeng* 79:348–353
- Chen C-L, Wu J-H, Liu W-T (2008) Identification of important microbial populations in the mesophilic and thermophilic phenol-degrading methanogenic consortia. *Water Res* 42:1963–1976
- Chen C, Yao X, Li QX, Wang Q, Liang J, Zhang S, Ming J, Liu Z, Deng J, Yoza BA (2018) Turf soil enhances treatment efficiency and performance of phenolic wastewater in an up-flow anaerobic sludge blanket reactor. *Chemosphere* 204:227–234
- Chou HH, Huang JS (2005) Comparative granule characteristics and biokinetics of sucrose-fed and phenol-fed UASB reactors. *Chemosphere* 59:107–116
- Dolfing J, Mulder JW (1985) Comparison of methane production rate and coenzyme f(420) content of methanogenic consortia in anaerobic granular sludge. *Appl Environ Microb* 49:1142–1145
- Fang H, Zhou G (1999) Degradation of phenol and p-cresol in anaerobic reactors. *Soc Sci Med* 117(5):142–149
- Fang HHP, Chen T, Li YY (1996) Degradation of phenol in wastewater in an upflow anaerobic sludge blanket reactor. *Water Res* 30(6):0–1360
- Fukuzaki S, Chang YJ, Nishio N (1991) Characteristics of granular Methanogenic sludge grown on lactate in a USAB reactor. *Ferment Bioeng* 72
- Gomez-Acata S, Esquivel-Rios I, Perez-Sandoval MV, Navarro-Noya Y, Rojas-Valdez A, Thalasso F, Luna-Guido M, Dendooven L (2017) Bacterial community structure within an activated sludge reactor added with phenolic compounds. *Appl Microbiol Biot* 101:3405–3414
- Goux X, Calusinska M, Lemaigre S, Marynowska M, Klocke M, Udelhoven T, Benizri E, Delfosse P (2015) Microbial community dynamics in replicate anaerobic digesters exposed sequentially to increasing organic loading rate, acidosis, and process recovery. *Biotechnol Biofuels* 8
- Heine-Dobbernack E, Schobert SM, Sahn H (1988) Relationship of intracellular coenzyme F(420) content to growth and metabolic activity of *Methanobacterium bryantii* and *Methanosarcina barkeri*. *Appl Environ Microb* 54:454–459
- Holmes DE, Shrestha PM, Walker DJF, Dang Y, Nevin KP, Woodard TL, Lovley DR (2017) Metatranscriptomic evidence for direct interspecies Electron transfer between *Geobacter* and *Methanotherox* species in Methanogenic Rice Paddy soils. *Appl Environ Microb* 83
- Hou J, Qiu Z, Han H, Zhang Q (2018) Toxicity evaluation of lignocellulose-derived phenolic inhibitors on *Saccharomyces cerevisiae* growth by using the QSTR method. *Chemosphere* 201: 286–293
- Huang X, Dong W, Wang H, Feng Y (2018) Role of acid/alkali-treatment in primary sludge anaerobic fermentation: insights into microbial community structure, functional shifts and metabolic output by high-throughput sequencing. *Bioresour Technol* 249:943–952
- Ju F, Zhang T (2014) Novel microbial populations in ambient and Mesophilic biogas-producing and phenol-degrading consortia unraveled by high-throughput sequencing. *Microbial Ecol* 68:235–246
- Ju F, Wang Y, Zhang T (2018) Bioreactor microbial ecosystems with differentiated methanogenic phenol biodegradation and competitive metabolic pathways unraveled with genome-resolved metagenomics. *Biotechnol Biofuels* 11
- Lai C-Y, Wen L-L, Zhang Y, Luo S-S, Wang Q-Y, Luo Y-H, Chen R, Yang X, Rittmann BE, Zhao H-P (2016) Autotrophic antimonate bio-reduction using hydrogen as the electron donor. *Water Res* 88: 467–474
- Leandro T, Rodriguez N, Rojas P, Sanz JL, da Costa MS, Amils R (2018) Study of methanogenic enrichment cultures of rock cores from the deep subsurface of the Iberian Pyritic Belt. *Heliyon* 4:e00605–e00605
- Li C, Tabassum S, Zhang Z (2014) An advanced anaerobic expanded granular sludge bed (AnaEG) for the treatment of coal gasification wastewater. *RSC Adv* 4:57580–57586
- Li Y, Tabassum S, Yu Z, Wu X, Zhang X, Song Y, Chua C, Zhang Z (2016) Effect of effluent recirculation rate on the performance of anaerobic bio-filter treating coal gasification wastewater under co-digestion conditions. *RSC Adv* 6:87926–87934
- Li Y, Sun Y, Li L, Yuan Z (2018a) Acclimation of acid-tolerant methanogenic propionate-utilizing culture and microbial community dissecting. *Bioresour Technol* 250:117–123
- Li Y, Tabassum S, Chu C, Zhang Z (2018b) Inhibitory effect of high phenol concentration in treating coal gasification wastewater in anaerobic biofilter. *J Environ Sci* 64:207–215
- Li Y, Ren C, Zhao Z, Yu Q, Zhao Z, Liu L, Zhang Y, Feng Y (2019) Enhancing anaerobic degradation of phenol to methane via solubilizing Fe (III) oxides for dissimilatory iron reduction with organic chelates. *Bioresour Technol* 291
- Liu Y, Zhu Y, Jia H, Yong X, Zhang L, Zhou J, Cao Z, Kruse A, Wei P (2017) Effects of different biofilm carriers on biogas production during anaerobic digestion of corn straw. *Bioresour Technol* 244: 445–451
- Loveland-Curtze J, Miteva V, Brenchley J (2010) Novel ultramicrobacterial isolates from a deep Greenland ice core represent a proposed new species, *Chryseobacterium greenlandense* sp nov. *Extremophiles* 14:61–69

- Luo J, Chen H, Han X, Sun Y, Yuan Z, Guo J (2017) Microbial community structure and biodiversity of size-fractionated granules in a partial nitrification-anammox process. *Fems Microbiol Ecol* 93
- Madigou C, Poirier S, Bureau C, Chapleur O (2016) Acclimation strategy to increase phenol tolerance of an anaerobic microbiota. *Bioresour Technol* 216:77–86
- McInerney MJ, Rohlin L, Mouttaki H, Kim U, Krupp RS, Rios-Hernandez L, Sieber J, Struchtemeyer CG, Bhattacharyya A, Campbell JW, Gunsalus RP (2007) The genome of *Syntrophus aciditrophicus*: life at the thermodynamic limit of microbial growth. *Proc Natl Acad Sci U S A* 104:7600–7605
- Muñoz Sierra JD, Lafita C, Gabaldon C, Spanjers H, van Lier JB (2017) Trace metals supplementation in anaerobic membrane bioreactors treating highly saline phenolic wastewater. *Bioresour Technol* 234:106–114
- Muñoz Sierra JDM, Oosterkamp MJ, Wang W, Spanjers H, van Lier JB (2018) Impact of long-term salinity exposure in anaerobic membrane bioreactors treating phenolic wastewater: performance robustness and endured microbial community. *Water Res* 141:172–184
- Na J-G, Lee M-K, Yun Y-M, Moon C, Kim M-S, Kim D-H (2016) Microbial community analysis of anaerobic granules in phenol-degrading UASB by next generation sequencing. *Biochem Eng J* 112:241–248
- Nguyen VK, Choi W, Park Y, Yu J, Lee T (2017) Characterization of diversified Sb(V)-reducing bacterial communities by various organic or inorganic electron donors. *Bioresour Technol* 250:239
- Nobu MK, Narihiro T, Liu M, Kuroda K, Mei R, Liu W-T (2017) Thermodynamically diverse syntrophic aromatic compound catabolism. *Environ Microbiol* 19:4576–4586
- Qian M, Li Y, Zhang Y, Sun Z, Wang Y, Feng J, Yao Z, Zhao L (2019) Efficient acetogenesis of anaerobic co-digestion of food waste and maize straw in a HSAD reactor. *Bioresour Technol* 283:221–228
- Ramakrishnan A, Gupta SK (2006) Anaerobic biogranulation in a hybrid reactor treating phenolic waste. *J Hazard Mater* 137:1488–1495
- Riviere D, Desvignes V, Pelletier E, Chaussonnerie S, Guermazi S, Weissenbach J, Li T, Camacho P, Sghir A (2009) Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *ISME J* 3:700–771
- Rosenkranz F, Cabrol L, Carballa M, Donoso-Bravo A, Cruz L, Ruiz-Filippi G, Chamy R, Lema JM (2013) Relationship between phenol degradation efficiency and microbial community structure in an anaerobic SBR. *Water Res* 47:6739–6749
- Satoru S, Akio U, Yoji I (2011) Microbial communities associated with acetate-rich gas-petroleum reservoir surface facilities. *Biosci Biotechnol Biochem* 75:1835–1837
- Shi S-L, Lv J-P, Liu Q, Nan F-R, Jiao X-Y, Feng J, Xie S-L (2018) Application of *Phragmites australis* to remove phenol from aqueous solutions by chemical activation in batch and fixed-bed columns. *Environ Sci Pollut R* 25:23917–23928
- Sierra JDM, Oosterkamp MJ, Wang W, Spanjers H, van Lier JB (2019) Comparative performance of upflow anaerobic sludge blanket reactor and anaerobic membrane bioreactor treating phenolic wastewater: overcoming high salinity. *Chem Eng J* 366:480–490
- Subramanyam R, Mishra IM (2013) Characteristics of methanogenic granules grown on glucose in an upflow anaerobic sludge blanket reactor. *Biosyst Eng* 114:113–123
- Sutton NB, Maphosa F, Morillo JA, Abu Al-Soud W, Langenhoff AAM, Grotenhuis T, Rijnaarts HHM, Smidt H (2013) Impact of Long-Term Diesel Contamination on Soil Microbial Community Structure. *Appl Environ Microb* 79:619–630
- Svensson K, Paruch L, Gaby JC, Linjordet R (2018) Feeding frequency influences process performance and microbial community composition in anaerobic digesters treating steam exploded food waste. *Bioresour Technol* 269:276–284
- Tay JH, He YX, Yan YG (2001) Improved anaerobic degradation of phenol with supplemental glucose. *J Environ Eng* 127(1):38–45
- Thauer RK, Kaster A-K, Seedorf H, Buckel W, Hedderich R (2008) Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Reviews Microbiology* 6:579–591
- Town JR, Links MG, Fonstad TA, Dumonceaux TJ (2014) Molecular characterization of anaerobic digester microbial communities identifies microorganisms that correlate to reactor performance. *Bioresour Technol* 151:249–257
- Veeresh GS, Kumar P, Mehrotra I (2005) Treatment of phenol and cresols in upflow anaerobic sludge blanket (UASB) process: a review. *Water Res* 39:154–170
- Viggi CC, Rossetti S, Fazi S, Paiano P, Majone M, Aulenta F (2014) Magnetite particles triggering a faster and more robust Syntrophic pathway of Methanogenic propionate degradation. *Environ Sci Technol* 48:7536–7543
- Wang J, Li Y (2016) Synergistic pretreatment of waste activated sludge using CaO₂ in combination with microwave irradiation to enhance methane production during anaerobic digestion. *Appl Energy* 183:1123–1132
- Wang W, Han H, Yuan M, Li H (2010) Enhanced anaerobic biodegradability of real coal gasification wastewater with methanol addition. *J Environ Sci* 22:1868–1874
- Wang W, Han H, Yuan M, Li H, Fang F, Wang K (2011a) Treatment of coal gasification wastewater by a two-continuous UASB system with step-feed for COD and phenols removal. *Bioresour Technol* 102:5454–5460
- Wang W, Ma W, Han H, Li H, Yuan M (2011b) Thermophilic anaerobic digestion of Lurgi coal gasification wastewater in a UASB reactor. *Bioresour Technol* 102:2441–2447
- Wang J, Liu H, Fu B, Xu K, Chen J (2013) Trophic link between syntrophic acetogens and homoacetogens during the anaerobic acidogenic fermentation of sewage sludge. *Biochem Eng J* 70:1–8
- Wang W, Wang S, Zhang J, Hu Z, Zhang X, Sierra JM (2016) Degradation kinetics of pentachlorophenol and changes in anaerobic microbial community with different dosing modes of co-substrate and zero-valent iron. *Int Biodeterior Biodegradation* 113:126–133
- Wang S, Hou X, Su H (2017a) Exploration of the relationship between biogas production and microbial community under high salinity conditions. *Sci Rep* 7
- Wang W, Wang S, Ren X, Hu Z, Yuan S (2017b) Rapid establishment of phenol- and quinoline-degrading consortia driven by the scoured cake layer in an anaerobic baffled ceramic membrane bioreactor. *Environ Sci Pollut R* 24:26125–26135
- Wang W, Wu B, Pan S, Yang K, Hu Z, Yuan S (2017c) Performance robustness of the UASB reactors treating saline phenolic wastewater and analysis of microbial community structure. *J Hazard Mater* 331:21–27
- Wei G, Yu J, Zhu Y, Chen W, Wang L (2008) Characterization of phenol degradation by *Rhizobium* sp CCNWTB 701 isolated from *Astragalus chrysopteru* in mining tailing region. *J Hazard Mater* 151:111–117
- Wu J, Afridi ZUR, Cao ZP, Zhang ZL, Poncin S, Li HZ, Zuo JE, Wang KJ (2016) Size effect of anaerobic granular sludge on biogas production: a micro scale study. *Bioresour Technol* 202:165–171
- Wu B, He C, Yuan S, Hu Z, Wang W (2019) Hydrogen enrichment as a bioaugmentation tool to alleviate ammonia inhibition on anaerobic digestion of phenol-containing wastewater. *Bioresour Technol* 276:97–102
- Xu H, Wang C, Yan K, Wu J, Zuo J, Wang K (2016) Anaerobic granule-based biofilms formation reduces propionate accumulation under high H₂ partial pressure using conductive carbon felt particles. *Bioresour Technol* 216:677–683
- Yan W, Sun F, Liu J, Zhou Y (2018) Enhanced anaerobic phenol degradation by conductive materials via EPS and microbial community alteration. *Chem Eng J* 352:1–9
- Yin Q, Yang S, Wang Z, Xing L, Wu G (2018) Clarifying electron transfer and metagenomic analysis of microbial community in the methane

- production process with the addition of ferrous oxide. *Chem Eng J* 333:216–225
- Zhang T, Ke SZ, Liu Y, Fang HP (2005) Microbial characteristics of a methanogenic phenol-degrading sludge. *Water Sci Technol* 52:73–78
- Zhang J, Li W, Lee J, Loh K-C, Dai Y, Tong YW (2017) Enhancement of biogas production in anaerobic co-digestion of food waste and waste activated sludge by biological co-pretreatment. *Energy* 137:479–486
- Zhang C, Yuan Q, Lu Y (2018a) Inhibitory effects of ammonia on syntrophic propionate oxidation in anaerobic digester sludge. *Water Res* 146:275–287
- Zhang F, Hou J, Miao L, Chen J, Xu Y, You G, Liu S, Ma J (2018b) Chlorpyrifos and 3,5,6-trichloro-2-pyridinol degradation in zero valent iron coupled anaerobic system: performances and mechanisms. *Chem Eng J* 353:254–263
- Zhao H, Zhu H, Ji Q, Yu G, Zhang Z (2013) Technological processes of hydrolytic acidification for coal gasification wastewater treatment. *Water Purif Technol* 32:45–50
- Zhao Z, Zhang Y, Holmes DE, Dang Y, Woodard TL, Nevin KP, Lovley DR (2016) Potential enhancement of direct interspecies electron transfer for syntrophic metabolism of propionate and butyrate with biochar in up-flow anaerobic sludge blanket reactors. *Bioresour Technol* 209:148–156

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.