

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

Accepted author manuscript

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.

[Click here to view linked References](#)

1 **Influence of particle size distribution on anaerobic degradation of phenol and analysis of**
2
3 **methanogenic microbial community**
4

5
6 Jing Wang^a, Benteng Wu^a, Julian Muñoz Sierra^{b,c}, Chunhua He^a, Zhenhu Hu^{a*}, Wei Wang^{a*}
7

8
9 ^aDepartment of Municipal Engineering, School of Civil Engineering, Hefei University of Technology,
10
11 Hefei, 230009, China
12

13
14 ^b Section Sanitary Engineering, Department of Water Management, Delft University of Technology,
15
16 Stevinweg 1, 2628 CN, Delft, The Netherlands.
17

18
19 ^c KWR Watercycle Research Institute, Groningenhaven 7, 3430 BB, Nieuwegein, The Netherlands.
20

21
22 The corresponding author, Email: wang_wei@hfut.edu.cn, dwhit@126.com, Tel., +86-551-62904144;
23

24
25 the co-corresponding author, Email: zhhu@hfut.edu.cn.
26
27

28
29
30
31 **Abstract:** Sludge morphology considerably affects the mechanism underlying microbial anaerobic
32
33 degradation of phenol. Here, we assessed the phenol degradation rate, specific methanogenic activity,
34
35 electron transport activity, coenzyme F₄₂₀ concentration, and microbial community structure of five
36
37 phenol-degrading sludge of varying particle sizes (i.e., <20, 20-50, 50-100, 100-200, and >200 μm).
38
39

40
41
42 The results indicated an increase in phenol degradation rate and microbial community structure that
43
44 distinctly correlated with an increase in sludge particle size. Although the sludge with the smallest
45
46 particle size (<20 μm) showed the lowest phenol degradation rate (9.3 mg COD·gVSS⁻¹ d⁻¹), its
47
48 methanogenic activity with propionic acid, butyric acid, and H₂/CO₂ as substrates, was the best, and the
49
50 concentration of coenzyme F₄₂₀ was the highest. The small particle size sludge did not contain
51
52 abundant syntrophic bacteria or hydrogenotrophic methanogens, but contained abundant acetoclastic
53
54 methanogens. Moreover, the floc sizes of the different sludge varied in important phenol-degrading
55
56
57
58
59
60

1 bacteria and archaea, which may dominate the synergistic mechanism. This study provides a new
2
3 perspective on the role of sludge floc size on the anaerobic digestion of phenol.
4
5

6 **Keywords:** anaerobic digestion; phenol degradation; particle size distribution; phenol-degrading
7
8 sludge; methanogenic activity; microbial community structure
9
10

11 **Acknowledgments**

12
13
14
15
16
17 This study was funded by the National Natural Science Foundation of China (51878232) and CAS
18
19 Key Laboratory of Urban Pollutant Conversion, University of Science and Technology of China
20
21 (KF201702).
22
23
24
25
26
27

28 **1. Introduction**

29
30
31 Phenolic compounds, naturally or artificially produced, have been identified as a major cause of
32
33 performance failure in anaerobic digesters (Rosenkranz et al. 2013). The concentration of phenol in the
34
35 effluents of coal gasification, coking, petrochemical manufacturing, and paper industries, was often as
36
37 high as $10\text{-}17 \times 10^3$ mg/L (Veeresh et al. 2005). Phenol has a toxic effect on all living organisms and
38
39 leads to protein denaturation and coagulation (Hou et al. 2018; Shi et al. 2018). Therefore, the removal
40
41 of phenol is essential for the protection of environment and human health. Currently, biodegradation of
42
43 phenol is generally preferred to physico-chemical methods of removal because of lower treatment costs
44
45 and a higher probability of complete mineralization. Phenol can be converted into methane under
46
47 anaerobic conditions making anaerobic degradation of phenol one of the most attractive and
48
49 cost-effective treatment methods (Ju et al. 2018; Ramakrishnan and Gupta 2006). For instance, the
50
51 concentration of phenol in coal gasification wastewater generally ranges from 500-3000 mg/L, and the
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 removal rate of phenol can reach 50-60% after anaerobic treatment (Wang et al. 2011b). Using upflow
2
3 anaerobic sludge blanket (UASB) treatment, the concentration of phenol was reduced to 110-250 mg/L,
4
5
6 with a hydraulic retention time (HRT) of 24 h (Wang et al. 2010, 2011a, b). Using anaerobic
7
8
9 sequencing batch reactor (ASBR) and anaerobic expanded granule sludge bed (EGSB) operations, with
10
11 a HRT of 48 h, the phenol concentration reduced to 110 and 140-160 mg/L, respectively (Li et al. 2014;
12
13
14 Zhao et al. 2013). However, high concentration of phenol frequently threatened the physical
15
16
17 morphology and microbial community structure of phenol-degrading bacteria and even led to
18
19
20 performance failure (Li et al. 2019).

21
22 In recent years, the granulation of sludge has successfully accelerated the start-up of anaerobic
23
24
25 reactors treating phenolic wastewater. Anaerobic granular sludge has high mass transfer resistance,
26
27
28 reducing the toxicity of phenol to microorganisms (Muñoz Sierra et al. 2017); however, some issues
29
30
31 with anaerobic granular sludge need to be overcome, such as long formation periods when the proper
32
33
34 seed is not available and strict operational parameter controls (Chen et al. 2018). Consequently,
35
36
37 anaerobic membrane bioreactors were employed to treat phenol-containing wastewater (Muñoz Sierra
38
39
40 et al. 2018; Wang et al. 2017b). It has previously been reported that the particle size of
41
42
43 phenol-degrading sludge shows a descending trend in anaerobic membrane bioreactors with excellent
44
45
46 performance (Muñoz Sierra et al. 2017). Li et al. (2018b) showed that with an increase in phenol
47
48
49 concentration in the anaerobic biofilter reactor, the removal rate of phenol reached a maximum value,
50
51
52 while the proportion of small particle size sludge gradually increased. It was also found that with a
53
54
55 decrease in particle size in the UASB reactor, phenol utilization rate increased (Sierra et al. 2019).
56
57
58 Interestingly, both the granular and small-flocculent sludge could achieve excellent removal of phenol
59
60
61 under optimum operational conditions. Although the composition of the microbial communities were
62
63
64
65

1 different under various phenolic wastewater treatment conditions, the phyla Chloroflexi, Thermotogae,
2
3 Cloacimonetes, Firmicutes, Proteobacteria, Synergistetes, and Euryarchaeaota were identified in every
4
5 phenol-degrading sludge (Wang et al. 2017c). Compared to the change in the abundance of other
6
7 bacteria, the abundance of the phylum Proteobacteria increased along with the size of sludge particles
8
9 (Chen et al. 2018), while the abundance of the genus *Methanosaeta* (belonging to the phylum
10
11 Euryarchaeaota) increased when the particle size decreased (Sierra et al. 2019). The morphology of the
12
13 sludge was clearly related to the metabolic characteristics and community structure of the
14
15 microorganisms (Huang et al. 2018); however, to the best of our knowledge, the mechanism underlying
16
17 phenol degradation in size-distributed phenol-degrading sludge has not been elucidated to date.
18
19
20
21
22
23
24

25 A summary of the information on anaerobic phenol-degrading sludge reported in the literature to
26
27 date is presented in Table 1. The variation in sludge morphology was observed in many studies on
28
29 anaerobic phenol-degrading reactors, but only a few of them examined the relationship between sludge
30
31 particle size and phenol degradation. In this study, phenol degradation rate and methanogenic activity
32
33 of size-distributed phenol-degrading sludge (i.e., <20, 20-50, 50-100, 100-200, and >200 μm) were
34
35 evaluated. Additionally, a comprehensive analysis of the microbial community structure of the
36
37 size-distributed phenol-degrading sludge was performed.
38
39
40
41
42
43
44

45 **Table 1**

46 **2 Materials and methods**

47 **2.1 Inoculums and experimental design**

48 The inoculum was obtained from a laboratory-scale UASB reactor treating synthetic phenolic
49
50 wastewater. The sludge was then sieved in 20, 50, 100, and 200 μm mesh to divide the samples into
51
52 five subsamples with different particle size ranges, namely, <20 μm , 20-50 μm , 50-100 μm , 100-200
53
54
55
56
57
58
59
60
61
62
63
64
65

1 µm, and >200 µm. The volatile suspended solids (VSS) of the five tested sized sludges were 0.20, 8.48,
2
3 15.96, 23.71 and 29.41 g/L, respectively. The total suspended solid (TSS) of each sludge was 3.01,
4
5
6 17.72, 26.47, 35.16, and 40.61 g/L, respectively. The experiment consisted of the following four parts:
7
8
9 (a) to determine the phenol degradation rate of each sludge; (b) to identify the methanogenic activity of
10
11 each sludge with different substrates, including acetate, propionate, butyric acid, and H₂/CO₂; (c) to
12
13 determine the potential electron transfer in each sludge based on coenzyme F₄₂₀ and electron transport
14
15 system activity; (d) to gain insight into the microbial community structure of the different sized
16
17 sludges.
18
19
20
21

22 2.2 Substrate utilization rate (SUR) and specific methanogenic activity (SMA) of sludge

23 To evaluate the activity of phenol degraders in each size-distributed sludge, the phenol utilization
24
25 rate was assessed. Batch tests were carried out in 250 mL serum bottles (with a working volume of
26
27 100 mL) and bottles were inoculated with different sized phenol-degrading sludge, 20 mg/L of phenol
28
29 and nutrient medium. The ratio of sludge mass (gVSS) to phenol COD (g) was 40:1 (Wang et al.
30
31 2017c). The volume of each sludge used per set of experiments was 50, 9.43, 5.01, 3.37, and 2.72 mL,
32
33 respectively (considering the low VSS concentration of the smallest particle size sludge, it was added
34
35 after concentration). The composition of the nutrient solution was as follows (in mg/L):
36
37 NaH₂PO₄·2H₂O, 62.40; K₂HPO₄·3H₂O, 136.95; NH₄Cl, 680; MgSO₄·7H₂O, 36; CaCl₂·2H₂O, 32;
38
39 FeCl₃·6H₂O, 0.8; MnCl₂·4H₂O, 0.2; CoCl₂·6H₂O, 0.8; CuCl₂·2H₂O, 0.012; ZnCl₂, 0.02; Na₂WO₄,
40
41 0.032; HBO₃, 0.02; Na₂SeO₃·5H₂O, 0.04; (NH₄)₆Mo₇O₂·4H₂O, 0.036; NiCl₂·6H₂O, 0.02. The bottles
42
43 were purged with N₂ for 1-2 min to create anaerobic conditions, then sealed with rubber stoppers and
44
45 cultivated at 35°C in a shaker at 140 rpm. Liquid samples were collected every 4 h with a syringe and
46
47 filtered through a 0.45 µm filter prior to liquid chromatography (LC) analysis. The phenol
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 concentration of the liquid samples was determined using high-performance liquid chromatography
2
3 (HPLC, 1260 Infinity, Agilent Inc., USA) with a C18 column (4.6 × 250 mm). The SUR of the sludge
4
5 was determined by calculating the slope of phenol reduction over time as $\text{mgCOD} \cdot \text{gVSS}^{-1} \text{d}^{-1}$. The
6
7 specific methanogenic activity (SMA) of each sludge was determined using the following four
8
9 substrates: acetate, propionate, butyric acid, and H_2/CO_2 (80/20 v/v), and samples were collected every
10
11
12 2 h. The procedure was repeated for phenol utilization rate batch tests. Methane content of biogas was
13
14 analyzed using a gas chromatograph (GC, SP-6890, Shandong Ruihong Ltd., China). The SMA of each
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

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.

2.3 Coenzyme F_{420} and electron transport system (ETS) activity tests

Sludge samples were collected after centrifugation at $16000 \times g$ 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 $95\text{-}100^\circ\text{C}$ for 30 min. Samples were
stirred every 10 min using a glass rod. Cooled samples were then centrifuged at $10000 \times g$ 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 ($10000 \times g$, 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

1 F₄₂₀ assay (Heine-Dobbernack et al. 1988).

2
3 Electron transport system activity was determined using 2-para
4 (iodo-phenyl)-3(nitrophenyl)-5(phenyl) tetrazolium chloride (INT) (Wang et al. 2016). For each
5
6 size-distributed sludge, 0.3 mL homogenized sludge sample was added to a 10 mL centrifuge tube and
7
8
9 treated as per the protocol devised by Wang et al. (2016). Data on F₄₂₀ and ETS of seed sludge are
10
11
12 calculated based on mass distribution. The electron transport activity was calculated as follows:
13
14
15

$$16 \quad U = 210.53 * \frac{D_{485}}{W} \quad (1)$$

17
18 Where U is the electron transfer system activity, D₄₈₅ is the absorbance at 485 nm, and W is the dry
19
20 weight of the sludge.
21
22

23 24 25 26 27 2.4 Analysis of microbial community structure

28
29 Samples were collected from each size-distributed sludge to analyze the bacterial and archaeal
30
31 community structure. Samples were analyzed by 16S rRNA gene high-throughput sequencing. The 16s
32
33 rRNA gene was amplified using the V3-V4 region universal primers 341F
34
35 (5-CCTACGGGNGGCWGCAG-3) and 805R (5-GACTACHVGGGTATCTAATCC-3). Samples were
36
37 subjected to high throughput sequencing using the Illumina Miseq platform (Illumina, Inc., San Diego,
38
39 CA, USA). The extraction and sequencing were then performed using Shanghai Sangon
40
41 Bioengineering Technology Service Co., Ltd pipeline. The read numbers of the five samples were
42
43 41685, 47076, 44998, 51827, and 46198, respectively, with the average read length being 416.20,
44
45 414.14, 417.34, 422.74, and 422.05, respectively. Coverage of all samples was 0.97. The measurement
46
47 could only be made after the quality met the requirements. Based on weighted UniFrac, principal
48
49 coordinate analysis (PCoA) was used to compare the similarity of different samples.
50
51
52
53
54
55
56
57
58

59 60 3 Results and discussion

3.1 Substrate utilization rates (SUR) of size-distributed phenol-degrading sludge

Fig.1

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 $\text{mgCOD}\cdot\text{gVSS}^{-1}\text{ d}^{-1}$), while the largest particle size sludge (>200 μm) had the highest phenol degradation rate (21.30 $\text{mgCOD}\cdot\text{gVSS}^{-1}\text{ d}^{-1}$). 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 $\text{mgCOD}\cdot\text{gVSS}^{-1}\text{ d}^{-1}$, respectively. The SUR of the seed sludge was 28.60 $\text{mgCOD}\cdot\text{gVSS}^{-1}\text{ d}^{-1}$. 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 was 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

1 shown to have a variety of dense bacteria throughout the sludge fractions, and the VFAs formed in the
2
3 sludge could easily be converted into methane by the surrounded methanogens (Chou and Huang 2005).
4
5
6 The internal arrangement of large particle size sludge may be beneficial for the conversion of substrates
7
8 and intermediates, and could create the best metabolic conditions for all its constituents (Subramanyam
9
10 et al. 2013). The >200 μm sludge contributed about fivefold more to the SUR of seed sludge than the
11
12 <20 μm sludge did, therefore, the structure of larger particle size phenol-degrading sludge accelerated
13
14 substrate uptake and resulted in a higher phenol degradation rate, as shown in Fig.1.
15
16
17
18

19 3.2 Specific methanogenic activity of size-distributed phenol-degrading sludge 20 21

22 **Fig.2**
23

24
25 The SMA of each phenol-degrading sludge with acetate, propionate, butyrate, and H_2/CO_2 as a
26
27 substrate, is shown in Fig. 2. The SMA-acetate value of sludge was enhanced with the increase of
28
29 sludge particle size, with the highest SMA-acetate value of $2.06 \pm 0.10 \text{ gCOD-CH}_4 \cdot \text{gVSS}^{-1} \text{ d}^{-1}$ in the
30
31 largest particle size sludge (>200 μm) (Fig 2a). Interestingly, the highest SMA-propionate,
32
33 SMA-butyrate, and SMA- H_2/CO_2 values, 0.72, 0.91, and $1.71 \text{ gCOD-CH}_4 \cdot \text{gVSS}^{-1} \text{ d}^{-1}$ respectively,
34
35 were obtained in the smallest particle size sludge (<20 μm). The SMA value of the seed sludge with
36
37 acetate, propionic acid, butyric acid, and H_2/CO_2 , was 1.72, 0.07, 0.51, and $0.38 \text{ gCOD-CH}_4 \cdot \text{gVSS}^{-1} \text{ d}^{-1}$,
38
39 respectively. The >200 μm sludge contributed about fourfold more to the SMA-acetate value of seed
40
41 sludge than the <20 μm sludge, while the <20 μm sludge contributed about 18 times more to the
42
43 SMA-propionic value of the seed sludge than the >200 μm sludge. The contribution of the <20 μm
44
45 and >200 μm sludge to the seed sludge SMA-butyric values were similar.
46
47
48
49
50
51
52
53
54

55
56 The reaction to convert butyric acid and propionic acid to acetate is energy consuming and cannot
57
58 be carried out under high hydrogen partial pressure conditions (Viggi et al. 2014; Zhang et al. 2018a).
59
60

1 The conversion rate of hydrogen to methane in the smallest particle size sludge (<20 μm) was, however,
2
3 very high, with the <20 μm sludge contributing about fivefold more to the SMA-H₂/CO₂ value of seed
4
5
6 sludge than the >200 μm sludge, indicating high hydrogen uptake and electronic uptake ability which
7
8 indirectly promoted the degradation rate of propionic and butyric acid. The SMA-H₂/CO₂ value of
9
10 large particle size sludge was relatively low. H₂/CO₂ was easily utilized by the methanogens in the
11
12 smallest particle size sludge, suggesting the likelihood of multiple degradation pathways for
13
14 accelerating the utilization rate of propionic, butyric acid, and H₂/CO₂, such as directly utilizing
15
16 hydrogen (Wang et al. 2017c), transforming hydrogen into acetic acid by homoacetogens (Wang et al.
17
18 2013a), or by syntrophic interactions (Zhao et al. 2016).

25 3.3 Potential electron transfer and coenzyme F₄₂₀ concentration of size-distributed phenol-degrading
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Fig.3

The electron transfer system activity and F₄₂₀ concentration of size-distributed phenol-degrading
sludge are presented in Fig. 3. Coenzyme F₄₂₀ presented a useful method to assess potential
methanogenic capacity, especially the H₂-utilizing capacity of the sludge (Yin et al. 2018). Although
the F₄₂₀ value of the seed sludge was only 0.28 μg·gVSS⁻¹, the concentration of F₄₂₀ in the smallest
particle size sludge (<20 μm) reached 2.51 μg·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
μg·mg⁻¹·d⁻¹). In previous studies, coenzyme F₄₂₀ 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 F₄₂₀ concentration tended to increase with the

1 sludge particle size, except at <20 μm , which was consistent with the tendency of SMA-acetate. The
2
3 F_{420} concentration in the smallest particle size sludge (<20 μm) was comparable to SMA- H_2/CO_2 ,
4
5
6 implying that coenzyme F_{420} was closely correlated to hydrogenotrophic activity. Liu et al. (2017) also
7
8
9 indicated that coenzyme F_{420} played an important role in the hydrogenotrophic pathway and was
10
11
12 required for the final reaction steps of the methanogenic pathway. In addition, the high F_{420}
13
14 concentration in the smallest particle size sludge correlated strongly the high utilization rate of
15
16
17 hydrogen and degradation rate of propionic and butyrate acid.
18
19

20 The highest ETS value ($12.17 \pm 1.00 \mu\text{g} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$) was observed in the 50-100 μm particle size
21
22 sludge (Fig. 3b), while the lowest ETS value ($2.99 \pm 0.17 \mu\text{g} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$) was found in the smallest particle
23
24 size sludge (<20 μm). ETS activity can be used to estimate the biological respiratory activity and
25
26
27 bioactivity of sludge (Yin et al. 2018; Zhang et al. 2018b). Syntrophic bacteria and methanogens can
28
29
30 achieve methanogenesis of phenol by extracellular electron transfer (Yan et al. 2018), however, the
31
32
33 electron transport system activity of the small particle size sludge was weak.
34
35

36 3.4 Microbial community structure of size-distributed phenol-degrading sludge

37 3.4.1 Bacteria

41 Fig.4

42
43
44 The results of principal coordinate analysis (PCoA) based on weighted unifrac distance of
45
46
47 bacterial communities in each of the five samples are shown in Fig. 4a. The smallest particle size
48
49
50 sludge (<20 μm) located far away from the other samples, indicating a significant difference in
51
52
53 bacterial community structure compared to the other particle size sludges. Similar bacterial community
54
55
56 structure was observed between the 100-200 μm and >200 μm particle size sludge, indicated by the
57
58
59 short unifrac distance between the two. Based on the PCoA results, we further identified the microbial
60
61
62
63
64
65

1 community structure at the phylum and genus levels, which are shown in Fig. 4b and c.

2
3 At the phylum level (Fig. 4b), Bacteroidetes (24.91%), Proteobacteria (17.32%), Thermotogae
4 (10.26%), Synergistetes (11.75%), Firmicutes (11%), Euryarchaeota (9.1%), and Chloroflexi (8.44%)
5
6 were dominant in the smallest particle size sludge. The relative abundance of the phyla Cloacimonetes
7 (3.8-15.37%) and Proteobacteria (19-34.29%) in larger particle size sludge was greater than that of the
8
9 smallest particle size sludge. The relative abundance of the phyla Bacteroidetes (24.91-5.47%) and
10
11 Euryarchaeota (9.1-0.27%) decreased with an increase in sludge particle size.
12
13
14
15
16
17
18
19

20 At the genus level (Fig. 4c), the relative abundances of the functional syntrophic phenol degraders
21
22 *Syntrophus* (2.27% to 29.12%), *Candidatus Cloacamonas* (1.49%-15.37%), and *Pelotomaculum*
23 (1.49% to 15.37%) increased along with particle size. The relative abundance of the syntrophic bacteria
24
25 *Mesotoga*, *Thermovirga*, and *Levilinea*, in all five particle sizes of sludge, was relatively high with no
26
27 significant difference between particle sizes. It has previously been reported that *Thermovirga*
28 (belonging to the phylum Synergistetes) has strong hydrolytic ability (Wang et al. 2017a). The role of
29
30 *Mesotoga* in anaerobic digestion has mainly been reported as a lactic acid utilizer (Goux et al. 2015),
31
32 but the exact role of *Mesotoga* in phenol degradation requires further exploration. *Levilinea* (belonging
33
34 to the phylum Chloroflexi) has often been found in phenolic treatment reactors. It is not only an
35
36 important hydrolytic fermentative bacterium (Antwi et al. 2017), but it is also a primary
37
38 acidogenic/acetogenic bacteria in anaerobic digesters (Zhang et al. 2017). The microbial phyla
39
40 Bacteroidetes, Firmicutes, Proteobacteria, and Chloroflexi have widely been reported in the anaerobic
41
42 treatment of phenolic wastewater (Li et al. 2016; Na et al. 2016). The phylum Proteobacteria plays an
43
44 important role in the key anaerobic digestion steps of hydrolysis and acetogenesis (Qian et al. 2019;
45
46 Wu et al. 2019). It has been reported that *Syntrophus* (belonging to the phylum Proteobacteria)
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 intracellularly degrades benzoate into acetate and shows higher substrate affinity to benzoate or higher
2
3 growth rate (Chen et al. 2008). Since the conversion of phenol to benzoate is a rate-limiting step, the
4
5 rapid consumption of benzoate promoted the forward reaction of phenol conversion. The sludge with
6
7 the smaller particle sizes of <20 µm and 20-50 µm had a relatively low abundance of *Syntrophus*,
8
9 indicating that the larger particle size sludges more than likely had the ability to convert phenol to
10
11 benzoate. It has been shown that *Syntrophus* establishes syntrophic interactions with hydrogenotrophic
12
13 methanogens through the H₂-producing fermentation of various organic matter (McInerney et al. 2007).
14
15 *Candidatus Cloacamonas*, belonging to the phylum Cloacimonetes, plays a putative role in the
16
17 metabolism of fatty acids and amino acids (Ju and Zhang 2014; Svensson et al. 2018). Similar to
18
19 *Syntrophus*, *Candidatus Cloacamonas* is also an important syntrophic bacterium, explaining why the
20
21 larger particle size sludge had the better phenol degradation rate. *Pelotomaculum*, belonging to the
22
23 phylum Firmicutes, has been often detected in reactors treating wastewater containing aromatics (Nobu
24
25 et al. 2017). Species belonging to the phylum Firmicutes are acidogens or hydrolytic bacteria, which
26
27 are conducive to the conversion of phenol to benzoate. Previously, a high phenol conversion rate has
28
29 been shown to correlate with a relatively high abundance of *Pelotomaculum* (Muñoz Sierra et al. 2018).
30
31 Chen et al. (2008) reported that *Desulfotomaculum* subcluster *Ih* contained *Pelotomaculum* spp., which
32
33 could convert phenol to benzoate at 30-37°C under anaerobic conditions. Our experimental data
34
35 showed the largest particle size sludge exhibited the highest phenol degradation rate, which correlated
36
37 to the highest relative abundance of *Pelotomaculum*. Furthermore, Na et al. (2016) stated that phenol
38
39 was degraded by *Syntrophus* and *Pelotomaculum*, both of which were dominant in the largest particle
40
41 size sludge and the high phenol degradation rate and SMA-acetate values could be attributed to their
42
43 presence.

1 Interestingly, only the smallest particle size sludge contained the bacteria *Rhizobium*, some strains
2
3 of which are known to efficiently recycle H₂ as an electron donor (Bretschger et al. 2015; Nguyen et al.
4
5 2017). Wei et al. (2008) and Gomez-Acata et al. (2017) reported that *Rhizobium* could utilize phenol as
6
7 a carbon source. Lai et al. (2016) found *Rhizobium* to be the predominant genus in H₂/CO₂-utilizing
8
9 experiments. Likewise, Leandro et al. (2018) found elevated *Rhizobium* in H₂/CO₂-fed cultures. It is,
10
11 therefore, obvious that only the smallest particle size sludge, with its relatively high abundance of
12
13 *Rhizobium* showed a high H₂/CO₂-utilizing rate, but the exact pathway through which *Rhizobium*
14
15 utilizes H₂ remains unclear and requires further investigations. As the degradation of butyric and
16
17 propionic acids is thermodynamically favorable under low partial hydrogen pressure (Xu et al. 2016),
18
19 the smallest particle size sludge might be efficient for utilizing butyric and propionic acid, as indicated
20
21 earlier. The genera *Chryseobacterium* (20.38%) and *Rhizobium* (2.85%) were only found in the
22
23 smallest particle size sludge (<20 μm). The genus *Chryseobacterium* has shown tolerance to a toxic
24
25 environment (Loveland-Curtze et al. 2010); thus, the smallest particle size sludge may play an
26
27 important role in resisting harsh conditions.

38 39 3.4.2 Archaea

42 Fig.5

44 The principal coordinate analysis (PCoA) based on weighted unifrac distance of archaea
45
46 communities in the five size samples is shown in Fig. 5a. Results show that the 50-100 μm, 100-200μm
47
48 and >200 μm particle size sludge have similar community structures. The seed sludge showed less
49
50 similarity in community structure compared to the other samples. Analysis of the archaeal microbial
51
52 community structure at the phylum and genus levels, based on the PCoA results, was conducted and is
53
54 shown in Fig. 5b and 5c. The main archaea from the five samples were found to consist of four genera,
55
56
57
58
59
60

1 of which three were hydrogenotrophic methanogens (*Methanofollis*, *Methanolinea*, and
2
3 *Methanosphaera*) and one was an acetoclastic methanogen (*Methanosaeta*). Most archaea in the smallest
4
5 particle size sludge (<20 µm) were principally acetoclastic methanogens with *Methanosaeta* (belonging
6
7 to the phylum Euryarchaeaota) accounting for 92.6% of the relative abundance. With an increase in
8
9 sludge particle size, the relative abundance of acetoclastic methanogens gradually decreased, and the
10
11 types of methanogens tended to vary. *Methanosaeta* plays a significant role in granule formation by
12
13 forming a skeleton within the granule to which other bacteria are able to attach (Sutton et al. 2013;
14
15 Thauer et al. 2008). Previous studies have shown that *Methanosaeta* conducts direct interspecies
16
17 electron transfer with other bacterial species, accelerating the symbiotic degradation of propionate and
18
19 butyrate (Holmes et al. 2017; Zhao et al. 2016). *Methanosaeta* has been noted for its electron
20
21 consumption capacity as an acetoclastic methanogen, especially the consumption of electrons released
22
23 from the oxidation of butyrate or propionate to acetate (Wang and Li 2016), which could explain why
24
25 the smallest particle size sludge had the highest SMA- butyrate and propionate values. In previously
26
27 described anaerobic digesters, members of the phyla Synergistetes (represented by *Syntrophus*) and
28
29 Firmicutes (represented by *Pelotomaculum*) were able to provide H₂ and short-chain acids through the
30
31 degradation of organic acids, which helped to establish syntrophic relationships with hydrogenotrophic
32
33 and acetoclastic methanogens (especially *Methanosaeta*) (Riviere et al. 2009; Zhang et al. 2005). As
34
35 mentioned in section 3.4.1 of this paper, *Candidatus Cloacimonas* could establish syntrophic
36
37 relationships with hydrogenotrophic methanogens. According to Fig.4b and Fig.5b, the smallest
38
39 particle size sludge showed less relative abundance of these syntrophic bacteria. Although the smallest
40
41 particle size sludge had high H₂-utilizing potential, according to the F₄₂₀ and SMA-H₂ values, it lacked
42
43 an effective syntrophic relationship. The smallest particle size sludge contained only a low abundance
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 of hydrogenotrophic methanogens and abundant acetoclastic methanogens; however, this sludge type
2
3 exhibited a strong ability to convert H₂/CO₂ to methane. Combined with the *Rhizobium* distribution, we
4
5 speculate that the smallest particle size sludge can convert hydrogen to acetic acid through hydrogen
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

of hydrogenotrophic methanogens and abundant acetoclastic methanogens; however, this sludge type exhibited a strong ability to convert H₂/CO₂ to methane. Combined with the *Rhizobium* distribution, we speculate that the smallest 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 samples. As previously discussed, *Methanosaeta* 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.

Fig.6

Table 2

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

1 each of those bacteria and archaea are reflected in Table 2. Fig.6 highlights some important bacteria
2
3 and archaea involved in anaerobic phenol degradation, especially syntrophic bacteria and methanogens.
4
5
6 All bacteria and archaea in Table 2 have been discussed in detail in the previous sections. In general,
7
8 compared to large granular sludge, especially the largest particle size sludge (>200 µm), the smallest
9
10 particle size sludge showed a poor variety of syntrophic bacteria, and lacked strong syntrophic
11
12 cooperation with methanogens.
13
14
15

16 **Conclusion**

17
18
19
20 Generally, in the sludge flocs, the smallest size part showed a higher rate in utilizing hydrogen and
21
22 propionic acid, compared to the larger size part, while the larger particle part exhibited a higher
23
24 utilization of acetate and phenol degradation. The larger particle size sludge also had abundant
25
26 syntrophic bacteria and the dominant genera in the smallest particle size sludge were mostly related to
27
28 hydrogen utilization and environment adaption. Phenol degradation is clearly a process of syntrophic
29
30 cooperation, substantiated by the sludge flocs showing the better phenol utilization ability than any of
31
32 the different sized sludge. Whatever the size of sludge flocs, the smallest size sludge is a beneficial
33
34 supplement to establish a strong syntrophic cooperation in the sludge flocs. This study has been
35
36 instrumental in elucidating the underlying microbial synergistic mechanisms in the sludge flocs and
37
38 their alterations related to the changes in sludge morphology.
39
40
41
42
43
44
45
46
47
48
49

50 **References**

- 51
52 Afridi ZUR, Wu J, Li ZH, Akand R, Cao ZP, Poncin S, Li HZ (2018) Novel insight of spatial mass
53 transfer conditions of upflow anaerobic reactor. J Clean Prod 204: 390-398
54
55 Antwi P, Li J, Boadi PO, Meng J, Shi E, Xue C, Zhang Y, Ayivi F (2017) Functional bacterial and
56 archaeal diversity revealed by 16S rRNA gene pyrosequencing during potato starch
57 processing wastewater treatment in an UASB. Bioresource Technol 235: 348-357
58
59 Bretschger O, Carpenter K, Phan T, Suzuki S, Ishii Si, Grossi-Soyster E, Flynn M, Hogan J (2015)
60
61
62
63
64
65

- 1 Functional and taxonomic dynamics of an electricity-consuming methane-producing microbial
2 community. *Bioresource Technol* 195: 254-264
- 3 Chang Y-J, Nishio N, Nagai S (1995) Characteristics of granular methanogenic sludge grown on
4 phenol synthetic medium and methanogenic fermentation of phenolic wastewater in a UASB
5 reactor. *Journal of Fermentation and Bioengineering* 79: 348-353
- 6
7 Chen C-L, Wu J-H, Liu W-T (2008) Identification of important microbial populations in the
8 mesophilic and thermophilic phenol-degrading methanogenic consortia. *Water Res* 42:
9 1963-1976
- 10
11 Chen C, Yao X, Li QX, Wang Q, Liang J, Zhang S, Ming J, Liu Z, Deng J, Yoza BA (2018) Turf soil
12 enhances treatment efficiency and performance of phenolic wastewater in an up-flow
13 anaerobic sludge blanket reactor. *Chemosphere* 204: 227-234
- 14
15 Chou HH, Huang JS (2005) Comparative granule characteristics and biokinetics of sucrose-fed and
16 phenol-fed UASB reactors. *Chemosphere* 59: 107-116
- 17
18 Dolfing J, Mulder JW (1985) Comparison of methane production rate and coenzyme f(420) content of
19 methanogenic consortia in anaerobic granular sludge. *Appl Environ Microb* 49: 1142-5
- 20
21 Fang H H P , Chen T , Li Y Y (1996) Degradation of phenol in wastewater in an upflow anaerobic
22 sludge blanket reactor. *Water Res* 30(6):0-1360.
- 23
24 Fang H , Zhou G (1999). Degradation of phenol and p-cresol in anaerobic reactors. *Social Science &*
25 *Medicine* 117(5):142-149.
- 26
27 Fukuzaki S , Chang Y J , Nishio N (1991). Characteristics of Granular Methanogenic Sludge Grown on
28 Lactate in a USAB Reactor. *Ferment Bioeng* 72.
- 29
30 Gomez-Acata S, Esquivel-Rios I, Perez-Sandoval MV, Navarro-Noya Y, Rojas-Valdez A, Thalasso F,
31 Luna-Guido M, Dendooven L (2017) Bacterial community structure within an activated
32 sludge reactor added with phenolic compounds. *Appl Microbiol Biot* 101: 3405-3414
- 33
34 Goux X, Calusinska M, Lemaigre S, Marynowska M, Klocke M, Udelhoven T, Benizri E, Delfosse P
35 (2015) Microbial community dynamics in replicate anaerobic digesters exposed sequentially
36 to increasing organic loading rate, acidosis, and process recovery. *Biotechnology for Biofuels*
37 8
- 38
39 Heine-Dobbernack E, Schoberth SM, Sahn H (1988) Relationship of Intracellular Coenzyme F(420)
40 Content to Growth and Metabolic Activity of *Methanobacterium bryantii* and *Methanosarcina*
41 *barkeri*. *Appl Environ Microb* 54: 454-9
- 42
43 Holmes DE, Shrestha PM, Walker DJF, Dang Y, Nevin KP, Woodard TL, Lovley DR (2017)
44 Metatranscriptomic Evidence for Direct Interspecies Electron Transfer between *Geobacter* and
45 *Methanotherox* Species in Methanogenic Rice Paddy Soils. *Appl Environ Microb* 83
- 46
47 Hou J, Qiu Z, Han H, Zhang Q (2018) Toxicity evaluation of lignocellulose-derived phenolic inhibitors
48 on *Saccharomyces cerevisiae* growth by using the QSTR method. *Chemosphere* 201: 286-293
- 49
50 Huang X, Dong W, Wang H, Feng Y (2018) Role of acid/alkali-treatment in primary sludge anaerobic
51 fermentation: Insights into microbial community structure, functional shifts and metabolic
52 output by high-throughput sequencing. *Bioresource Technol* 249: 943-952
- 53
54 Ju F, Zhang T (2014) Novel Microbial Populations in Ambient and Mesophilic Biogas-Producing and
55 Phenol-Degrading Consortia Unraveled by High-Throughput Sequencing. *Microbial Ecol* 68:
56 235-246
- 57
58 Ju F, Wang Y, Zhang T (2018) Bioreactor microbial ecosystems with differentiated methanogenic
59 phenol biodegradation and competitive metabolic pathways unraveled with genome-resolved
60

- 1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- 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 Advances* 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 Advances* 6: 87926-87934
- Li Y, Sun Y, Li L, Yuan Z (2018a) Acclimation of acid-tolerant methanogenic propionate-utilizing culture and microbial community dissecting. *Bioresource 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. *Bioresource 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. *Bioresource 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. *Bioresource 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. *Proceedings of the National Academy of Sciences of the United States of America* 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. *Bioresource 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. *Bioresource Technol*

- 1 Nobu MK, Narihiro T, Liu M, Kuroda K, Mei R, Liu W-T (2017) Thermodynamically diverse
2 syntrophic aromatic compound catabolism. *Environ Microbiol* 19: 4576-4586
3
- 4 Qian M, Li Y, Zhang Y, Sun Z, Wang Y, Feng J, Yao Z, Zhao L (2019) Efficient acetogenesis of
5 anaerobic co-digestion of food waste and maize straw in a HSAD reactor. *Bioresource*
6 *Technol* 283: 221-228
7
- 8 Ramakrishnan A, Gupta SK (2006) Anaerobic biogranulation in a hybrid reactor treating phenolic
9 waste. *J Hazard Mater* 137: 1488-1495
10
- 11 Rosenkranz F, Cabrol L, Carballa M, Donoso-Bravo A, Cruz L, Ruiz-Filippi G, Chamy R, Lema JM
12 (2013) Relationship between phenol degradation efficiency and microbial community
13 structure in an anaerobic SBR. *Water Res* 47: 6739-6749
14
- 15 Riviere D, Desvignes V, Pelletier E, Chaussonnerie S, Guermazi S, Weissenbach J, Li T, Camacho P,
16 Sghir A (2009) Towards the definition of a core of microorganisms involved in anaerobic
17 digestion of sludge. *Isme Journal* 3: 700-71
18
- 19 Satoru S, Akio U, Yoji I (2011) Microbial communities associated with acetate-rich gas-petroleum
20 reservoir surface facilities. *Bioscience Biotechnology & Biochemistry* 75: 1835-1837
21
- 22 Shi S-L, Lv J-P, Liu Q, Nan F-R, Jiao X-Y, Feng J, Xie S-L (2018) Application of *Phragmites australis*
23 to remove phenol from aqueous solutions by chemical activation in batch and fixed-bed
24 columns. *Environ Sci Pollut R* 25: 23917-23928
25
- 26 Sierra JDM, Oosterkamp MJ, Wang W, Spanjers H, van Lier JB (2019) Comparative performance of
27 upflow anaerobic sludge blanket reactor and anaerobic membrane bioreactor treating phenolic
28 wastewater: Overcoming high salinity. *Chem Eng J* 366: 480-490
29
- 30 Subramanyam, Revanuru, Mishra IM (2013) Characteristics of methanogenic granules grown on
31 glucose in an upflow anaerobic sludge blanket reactor. *Biosystems Engineering* 114: 113-123
32
- 33 Svensson K, Paruch L, Gaby JC, Linjordet R (2018) Feeding frequency influences process
34 performance and microbial community composition in anaerobic digesters treating steam
35 exploded food waste. *Bioresource Technol* 269: 276-284
36
- 37 Tay J H , He Y X , Yan Y G (2001). Improved Anaerobic Degradation of Phenol with Supplemental
38 Glucose. *J Environ Eng* 127(1):38-45.
39
- 40 Town JR, Links MG, Fonstad TA, Dumonceaux TJ (2014) Molecular characterization of anaerobic
41 digester microbial communities identifies microorganisms that correlate to reactor
42 performance. *Bioresource Technol* 151: 249-257
43
- 44 Veeresh GS, Kumar P, Mehrotra I (2005) Treatment of phenol and cresols in upflow anaerobic sludge
45 blanket (UASB) process: a review. *Water Res* 39: 154-170
46
- 47 Viggli CC, Rossetti S, Fazi S, Paiano P, Majone M, Aulenta F (2014) Magnetite Particles Triggering a
48 Faster and More Robust Syntrophic Pathway of Methanogenic Propionate Degradation.
49 *Environ Sci Technol* 48: 7536-7543
50
- 51 Wang J, Liu H, Fu B, Xu K, Chen J (2013a) Trophic link between syntrophic acetogens and
52 homoacetogens during the anaerobic acidogenic fermentation of sewage sludge. *Biochem Eng*
53 *J* 70: 1-8
54
- 55 Wang J, Li Y (2016) Synergistic pretreatment of waste activated sludge using CaO₂ in combination
56 with microwave irradiation to enhance methane production during anaerobic digestion. *Appl*
57 *Energ* 183: 1123-1132
58
- 59 Wang S, Hou X, Su H (2017a) Exploration of the relationship between biogas production and
60
61
62
63
64
65

- microbial community under high salinity conditions. *Scientific Reports* 7
- 1 Wang W, Han H, Yuan M, Li H (2010) Enhanced anaerobic biodegradability of real coal gasification
2 wastewater with methanol addition. *J Environ Sci* 22: 1868-1874
- 3
4 Wang W, Han H, Yuan M, Li H, Fang F, Wang K (2011a) Treatment of coal gasification wastewater
5 by a two-continuous UASB system with step-feed for COD and phenols removal. *Bioresource*
6 *Technol* 102: 5454-5460
- 7
8 Wang W, Ma W, Han H, Li H, Yuan M (2011b) Thermophilic anaerobic digestion of Lurgi coal
9 gasification wastewater in a UASB reactor. *Bioresource Technol* 102: 2441-2447
- 10
11 Wang W, Wang S, Zhang J, Hu Z, Zhang X, Sierra JM (2016) Degradation kinetics of
12 pentachlorophenol and changes in anaerobic microbial community with different dosing
13 modes of co-substrate and zero-valent iron. *Int Biodeter Biodegr* 113: 126-133
- 14
15 Wang W, Wang S, Ren X, Hu Z, Yuan S (2017b) Rapid establishment of phenol- and
16 quinoline-degrading consortia driven by the scoured cake layer in an anaerobic baffled
17 ceramic membrane bioreactor. *Environ Sci Pollut R* 24: 26125-26135
- 18
19 Wang W, Wu B, Pan S, Yang K, Hu Z, Yuan S (2017c) Performance robustness of the UASB reactors
20 treating saline phenolic wastewater and analysis of microbial community structure. *J Hazard*
21 *Mater* 331: 21-27
- 22
23 Wei G, Yu J, Zhu Y, Chen W, Wang L (2008) Characterization of phenol degradation by *Rhizobium* sp
24 CCNWTB 701 isolated from *Astragalus chrysopteru* in mining tailing region. *J Hazard Mater*
25 151: 111-117
- 26
27 Wu B, He C, Yuan S, Hu Z, Wang W (2019) Hydrogen enrichment as a bioaugmentation tool to
28 alleviate ammonia inhibition on anaerobic digestion of phenol-containing wastewater.
29 *Bioresource Technol* 276: 97-102
- 30
31 Wu J, Afridi ZUR, Cao ZP, Zhang ZL, Poncin S, Li HZ, Zuo JE, Wang KJ (2016) Size effect of
32 anaerobic granular sludge on biogas production: A micro scale study. *Bioresource Technol*
33 202: 165-171
- 34
35 Xu H, Wang C, Yan K, Wu J, Zuo J, Wang K (2016) Anaerobic granule-based biofilms formation
36 reduces propionate accumulation under high H₂ partial pressure using conductive carbon felt
37 particles. *Bioresource Technol* 216: 677-683
- 38
39 Yan W, Sun F, Liu J, Zhou Y (2018) Enhanced anaerobic phenol degradation by conductive materials
40 via EPS and microbial community alteration. *Chem Eng J* 352: 1-9
- 41
42 Yin Q, Yang S, Wang Z, Xing L, Wu G (2018) Clarifying electron transfer and metagenomic analysis
43 of microbial community in the methane production process with the addition of ferrous
44 oxide. *Chem Eng J* 333: 216-225
- 45
46 Zhang C, Yuan Q, Lu Y (2018a) Inhibitory effects of ammonia on syntrophic propionate oxidation in
47 anaerobic digester sludge. *Water Res* 146: 275-287
- 48
49 Zhang F, Hou J, Miao L, Chen J, Xu Y, You G, Liu S, Ma J (2018b) Chlorpyrifos and
50 3,5,6-trichloro-2-pyridinol degradation in zero valent iron coupled anaerobic system:
51 Performances and mechanisms. *Chem Eng J* 353: 254-263
- 52
53 Zhang J, Li W, Lee J, Loh K-C, Dai Y, Tong YW (2017) Enhancement of biogas production in
54 anaerobic co-digestion of food waste and waste activated sludge by biological
55 co-pretreatment. *Energy* 137: 479-486
- 56
57 Zhang T, Ke SZ, Liu Y, Fang HP (2005) Microbial characteristics of a methanogenic phenol-degrading
58 sludge. *Water Sci Technol* 52: 73-78
- 59
60
61
62
63
64
65

1 Zhao H., Zhu H., Ji Q., Yu G., Zhang Z (2013) Technological processes of hydrolytic acidification for
2 coal gasification wastewater treatment. *Water Purif. Technol* 32: 45-50
3 Zhao Z, Zhang Y, Holmes DE, Dang Y, Woodard TL, Nevin KP, Lovley DR (2016) Potential
4 enhancement of direct interspecies electron transfer for syntrophic metabolism of propionate
5 and butyrate with biochar in up-flow anaerobic sludge blanket reactors. *Bioresource Technol*
6 209: 148-156
7
8
9

10 11 12 13 14 15 16 17 **Figure captions**

18
19
20 Fig.1 Substrate utilization rate (SUR) of size-distributed phenol-degrading sludge
21

22
23 Fig.2 Specific methanogenic activity (SMA) of size-distributed phenol-degrading sludge
24

25
26 Fig.3 F₄₂₀ (a) and ETS activity (b) of size-distributed phenol-degrading sludge
27

28
29 Fig.4 (a) PCoA based on weighted unifrac distance of bacterial communities in size-distributed
30 phenol-degrading sludge; Relative abundances of the bacteria in the at the (b) phylum level, (c) genus
31
32
33
34 level
35

36
37 Fig.5 (a) PCoA based on weighted unifrac distance of archaea communities in size-distributed
38
39 phenol-degrading sludge; Relative abundances of the archaea in the at the (b) phylum level, (c) genus
40
41
42 level that relative abundance below 1%.
43

44
45 Fig.6 Phenol degradation pathway with related bacteria and archaea
46

47 **Table captions**

48
49
50 Table 1 Information of anaerobic phenol-degrading sludge reported in the literatures
51

52
53 Table 2 The related bacteria and archaea and their relative abundance
54

54 55 **Supplementary material**

56
57 Fig. S1 Phenol concentration change of size-distributed phenol-degrading sludge in substrate utilization
58
59
60 rate (SUR) experiment.
61

Figure 1

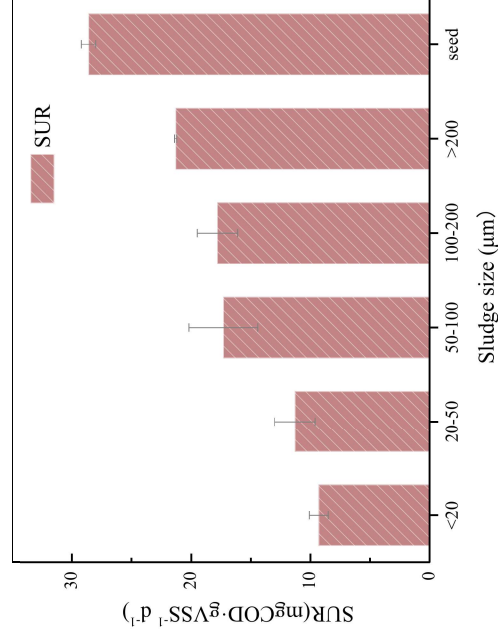


Fig.1 Substrate utilization rate (SUR) of size-distributed phenol-degrading sludge ('seed' represents the seed sludge without any size segregation; '<20, 20-50, 50-100, 100-200 and >200' represent the sludge samples with particle size ranges of <20µm, 20-50µm, 50-100 µm, 100-200 µm, and >200 µm, respectively)

Figure 2

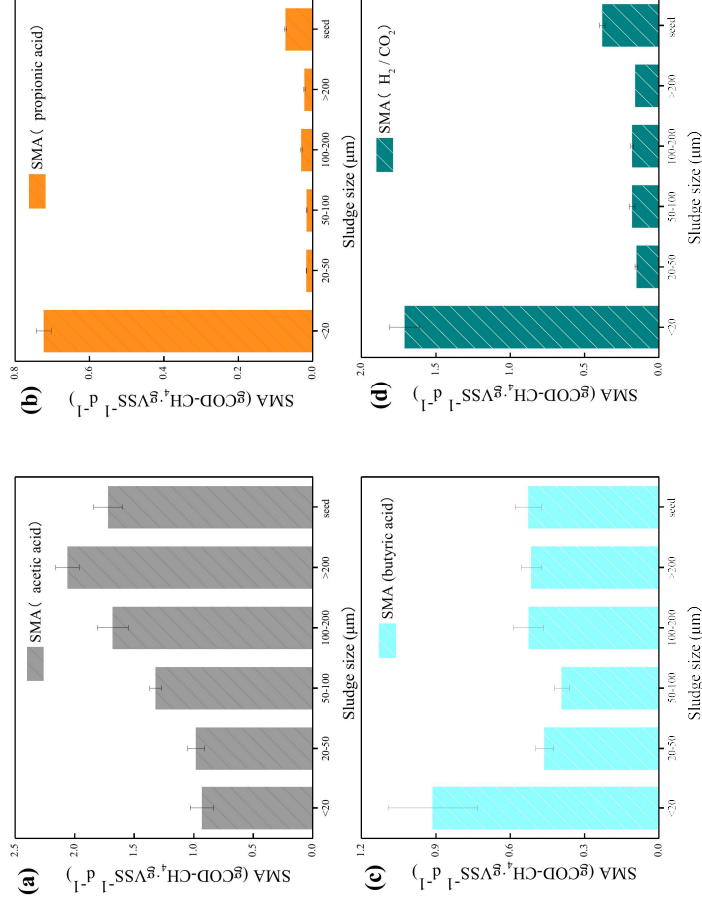


Fig.2 Specific methanogenic activity (SMA) of size-distributed phenol-degrading sludge. ((a)-(d)

represent 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 <20µm, 20-50µm, 50-100 µm, 100-200 µm, and >200 µm, respectively)

Figure 3

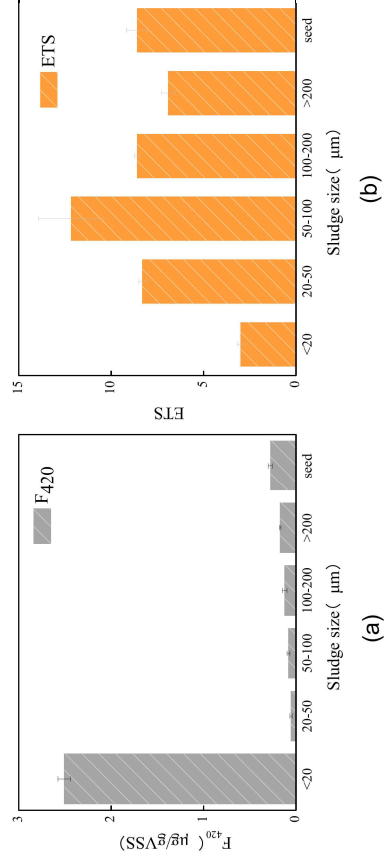


Fig.3 F₄₂₀ (a) and ETS activity (b) of size-distributed phenol-degrading sludge. (F₄₂₀ represents the concentration of coenzyme F₄₂₀; ETS represents the electron transfer system, 'seed' represents the seed sludge without any size segregation; '<20, 20-50, 50-100, 100-200 and >200' represent the sludge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

Figure 4

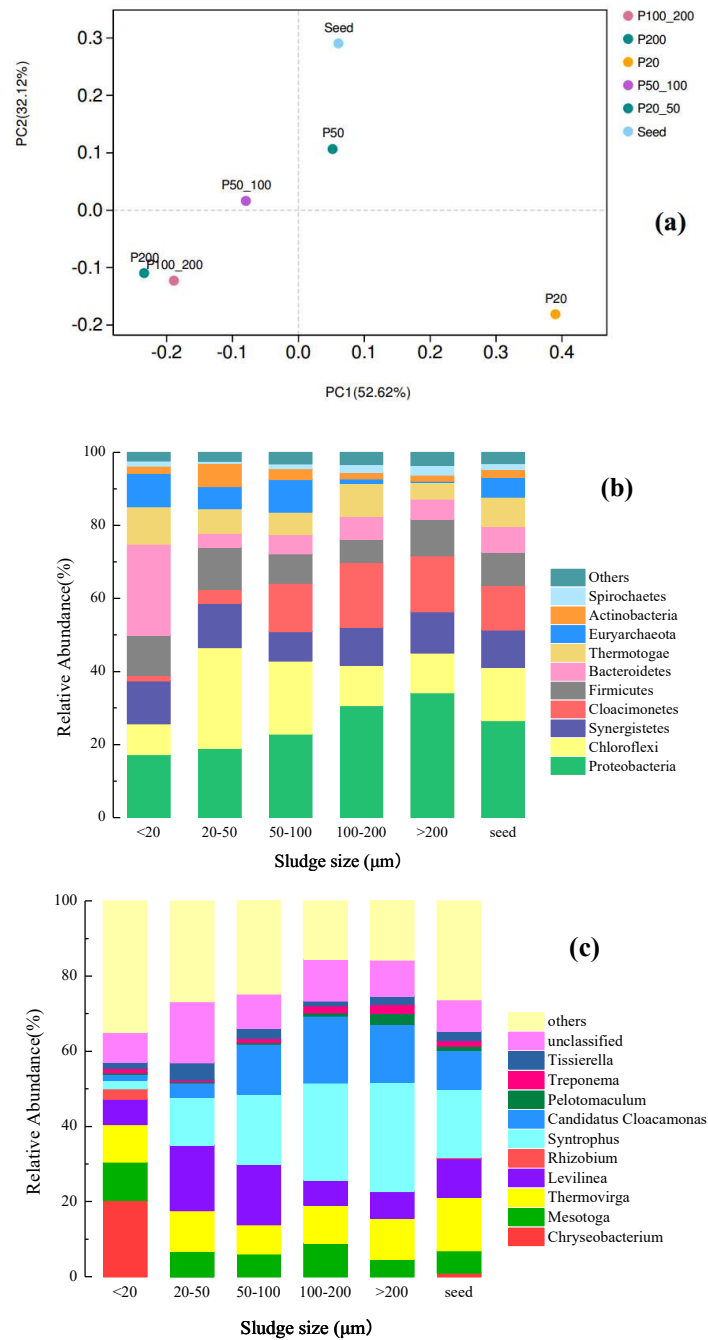


Fig.4 (a) PCoA based on weighted unfrac distance of bacterial communities in size-distributed phenol-degrading sludge; Relative abundances of the bacteria in the at the (b) phylum level, (c) genus level (“Other” represents all classified taxa that were <1% in all samples. PCoA represents Principal coordinates 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 μ m, 20-50 μ m, 50-100 μ m, 100-200 μ m, >200 μ m and the seed sludge without any size segregation, respectively)

Figure 5

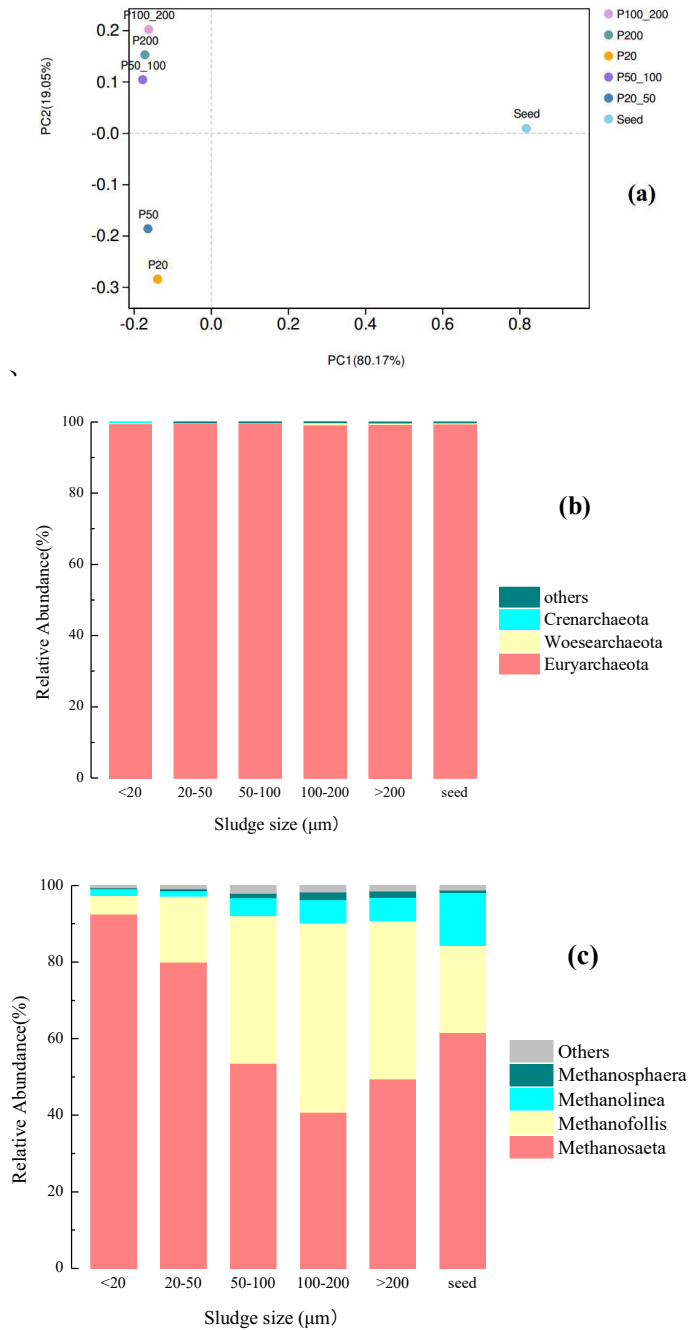


Fig.5 (a) PCoA based on weighted unifracs distance of archaea communities in size-distributed phenol-degrading sludge; Relative abundances of the archaea in the at the (b) phylum level, (c) genus level (“Other” represents all classified taxa that were <1% in all samples. PCoA represents Principal coordinates 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 μ m, 20-50 μ m, 50-100 μ m, 100-200 μ m, >200 μ m

and the seed sludge without any size segregation, respectively)

Figure 6

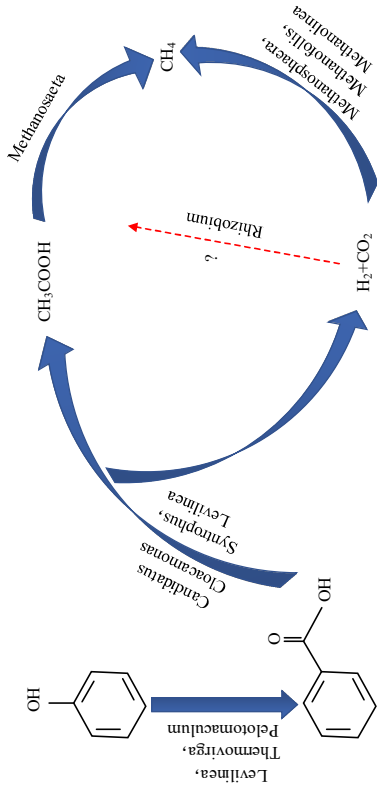


Fig.6 Phenol degradation pathway with related bacteria and archaea

Table 1

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

* represents the median particle size median size (D50). The symbol '-' represents this information was not mentioned in the literature. 'UASB' represents up-flow anaerobic sludge blanket reactor. 'AnMBR' represents anaerobic membrane bioreactor. 'ABCMBR' represents anaerobic baffled ceramic membrane bioreactor.

Table 2

Table 2 The related bacteria and archaea and their relative abundance

Type	Name	Relative abundance (%)						seed
		<20µm	20-50µm	50-100µm	100-200µm	>200µm		
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</i>	1.49	3.8	13.25	17.76	15.37	10.31	
	<i>Cloacamonas</i>							
Archaea	<i>Rhizobium</i>	2.85	0.03	0.00	0.00	0.00	0.42	
	<i>Methanoseta</i>	92.65	80.09	53.64	40.89	49.52	61.75	
	<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	