Effect of the dosage of ferroferric oxide on batch anaerobic treatment of high strengthen synthetic wastewater

Qidong Yin*, Zhenhu Hu**, Yuepeng Sun*, Bo Li*, Guangxue Wu*,†

* Key Laboratory of Microorganism Application and Risk Control (MARC) of Shenzhen, Graduate School at Shenzhen, Tsinghua University, Shenzhen, 518055, Guangdong, China
(†Corresponding author, E-mail: wu.guangxue@sz.tsinghua.edu.cn)
** School of Civil Engineering, Hefei University of Technology, Hefei 230009, China

Abstract
Direct interspecies electron transfer (DIET) plays an important role in anaerobic treatment of wastewater or anaerobic digestion of solids waste, and conductive materials may enhance DIET in these processes. Anaerobic microbial communities were initially acclimated with protein or carbohydrate. Then, the effect of dosage of ferroferric oxide (Fe_3O_4) on anaerobic treatment of synthetic wastewater was examined in batch experiments. For the anaerobic microorganisms acclimated with protein-based substrate, during anaerobic treatment, the lag phase was shortened and the CH_4 production rate was increased with the addition of Fe_3O_4. For the anaerobic sludge acclimated with starch-based substrate, during anaerobic treatment, the addition of Fe_3O_4 had little effect on the CH_4 production while the acetic acid concentration was high. The effect of dosage of Fe_3O_4 on hydrolysis/acidification of protein and on methanogenesis of acetate showed that it had little effect on these two processes. *Methanosarcina* (66.28%) and *Methanosaeta* (19.56%) were dominant methanogens in the sludge acclimated with tryptone and these methanogens were proved to accept electrons via DIET. While *Methanobacterium* (92.80%) was predominant in the sludge acclimated with starch. Therefore, the effect of Fe_3O_4 on anaerobic treatment was due to its improvement of interrelationship between hydrolysis/acidification and methanogenesis, which was also affected by the organic carbon acclimated microbial communities.

Keywords
Ferroferric oxide; methanogenesis; hydrolysis/acidification; direct interspecies electron transfer

INTRODUCTION

Anaerobic treatment is widely used for treatment of wastewater with high concentrations of organic carbon. Its contribution to both pollution control and energy recovery, making it one of sustainable technologies for wastewater management. Interspecies electron transfer via H_2/formate plays a key role in methane production by methanogens. Recently, direct interspecies electron transfer (DIET) has been demonstrated to be another mechanism for electron transfer (Lovley, 2011). During DIET, microorganisms must possess the ability to exchange electrons through biological electrical connections. On the other hand, the use of conductive material is believed to facilitate DIET for syntrophic methane (CH_4) production, such as granular active carbon (GAC), carbon cloth, carbon nanotube, and conductive iron oxides (Liu et al., 2012; Chen, 2014; Li et al., 2015a; Li et al., 2015b, Zhuang et al., 2015).

These conductive materials function as electron conduits between acidogenic bacteria and methanogens, accelerating the CH_4 production rate and shortening the lag phase of CH_4 production. Organic substrates such as ethanol, acetate, butyrate and propionate were used as carbon sources to examine the facilitation of conductive materials on anaerobic treatment systems or co-cultures between methanogens and acidogenic bacteria (Liu et al., 2012; Li et al., 2015a; Zhuang et al., 2015; Yamada et al., 2015; Zhu et al., 2015). But so far, only *Geobacter*, *Methanosarcin*a and *Methanosaeta* were proved to be capable of transferring or accepting electrons via DIET, and pili or c-type cytochrome was shown to responsible for the extracellular electron transfer (Rotaru et al., 2014a; Morita et al., 2011; Shrestha et al., 2014). However, complicate organic substrates were less investigated and the ability of extracellular electron transfer from other acidogenic bacteria and

archaea remains unknown.

In this study, ferroferric oxide (Fe_3O_4) was chosen as the conductive material to examine its effect on the performance of anaerobic treatment of high strength wastewater. Furthermore, the effect of organic substrates on anaerobic treatment with the dosage of conductive materials, i.e., starch and tryptone, were chosen to represent typical organic substrates of carbohydrate and protein, respectively. Finally, microbial community of the anaerobic treatment system was also analysed to examine its effect on DIET.

MATERIALS AND METHODS

Anaerobic sludge acclimation

Anaerobic sludge was taken from two lab-scale anaerobic reactors fed with tryptone or starch, which had been continuously operated at 35°C with a hydraulic retention time of 48 h and a volumetric chemical oxygen demand (COD) loading rate of 1500 mg/(L·d). The components of fed wastewater were as follows: 290 mg/L NH_4Cl, 100 mg/L $CaCl_2$, 200 mg/L $MgCl_2$, 70 mg/L Na_2HPO_4, 200 mg/L $KHCO_3$ and 1 mL/L trace elements. Trace elements consisted of 1 g/L $FeCl_2 \cdot 4H_2O$, 100 mg/L $CoCl_2 \cdot 6H_2O$, 200 mg/L $NiCl_2 \cdot 6H_2O$, 100 mg/L $MnCl_2 \cdot 4H_2O$, 100 mg/L $NaMoO_4 \cdot 2H_2O$, 100 mg/L H_3BO_3, 100 mg/L $NaWO_4 \cdot 2H_2O$ and 100 mg/L $NaSeO_3$.

Batch experiments

After these two reactors reached steady state, the following experiments were carried out: (i) Effect of Fe_3O_4 on methanogenesis of tryptone or starch, (ii) Effect of various Fe_3O_4 concentrations on methanogenic degradation of tryptone or starch, (iii) Effect of Fe_3O_4 on hydrolysis and acidification of tryptone, and (iv) Effect of Fe_3O_4 on methane producing phase.

For experiment (i), tryptone or starch was used as substrate and 10 g/L Fe_3O_4 was dosed in the Fe_3O_4 group. For experiment (ii), tryptone or starch was used as substrate and 0 g/L, 2.5 g/L, 5 g/L, 10 g/L, 15 g/L and 20 g/L Fe_3O_4 was add into each bottle (noted as Control, F2.5, F5, F10, F15, F20) to evaluate the effect of Fe_3O_4 concentrations on anaerobic treatment. For experiment (iii), tryptone was used as substrate and 10 g/L Fe_3O_4 was dosed in the Fe_3O_4 group. During the study, 10 mmol/L 2-bromoethanesulfonic acid sodium salt (BES) was dosed to inhibit the activity of methanogens. For experiment (iv), NaAc was used as the solo carbon source to prevent haydrolytic acidification and 10 g/L Fe_3O_4 was dosed in the Fe_3O_4 group.

All batch experiments were carried out in 500 mL saline bottles with 200 mL anaerobic sludge, 300 mL nutrient solution and 0.5 mL of trace element. Before the experiments, nitrogen gas (N_2) was used to remove the oxygen from the headspace of the reactors for 3 min, and then bottles were sealed with rubber stoppers and mixed in an air bath shaker at 170 r/min and 35°C. The suspended solids (SS) and volatile suspended solids (VSS) concentrations of these experiments were 2.39±0.22 g/L and 2.04±0.21 g/L. Liquid and gas samples were periodically collected to analyse concentrations of COD, volatile fatty acids (VFAs) and CH_4, respectively.

Analytical methods

COD, SS and VSS were measured according to standard methods (APHA, 1995). Soluble COD (SCOD) was measured after the samples filtered through 0.45 μm filter membranes.

CH_4 was measured by a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a thermal conductivity detector and a 2 m packed column (Porapak N). The temperatures of injector, detector and column were kept at 90, 100 and 35°C, respectively. Helium gas was used as the carrier gas at a flow rate of 25 mL/min. The modified Gompertz model (Zwietering et al., 1990) was used to

analyze the kinetic parameters of methane production which included ultimate CH_4 yield (P_{max}), maximum CH_4 production rate (R_{max}) and lag phase (λ).

VFAs were tested by a gas chromatograph (GC-2014, Shimadzu, Japan) equipped with a flame ionization detector and a capillary column. The carrier gas was N_2 at a flow rate of 50 mL/min, with a split ratio of 15 at a flow rate of 1.1 mL/min in the column and a purge flow rate of 3.0 mL/min. The oven temperature was increased proportionally from 70°C to 200°C within 10 min and the final holding duration was 2 min. The temperatures of both injector and detector were 240°C. The injected volume of the pre-acidified samples (adjust the pH to below 3 with formic acid) was 1 μL.

DNA was extracted by PowerSoil DNA extraction kit, and then microbial communities were analyzed by the 16S rRNA high-throughput sequencing method (Guo et al., 2012).

RESULTS

Effect of Fe_3O_4 on methanogenesis of tryptone or starch

Fig. 1 shows the result of batch experiments with or without the dosage of Fe_3O_4 on anaerobic treatment with organic carbon of tryptone or starch.

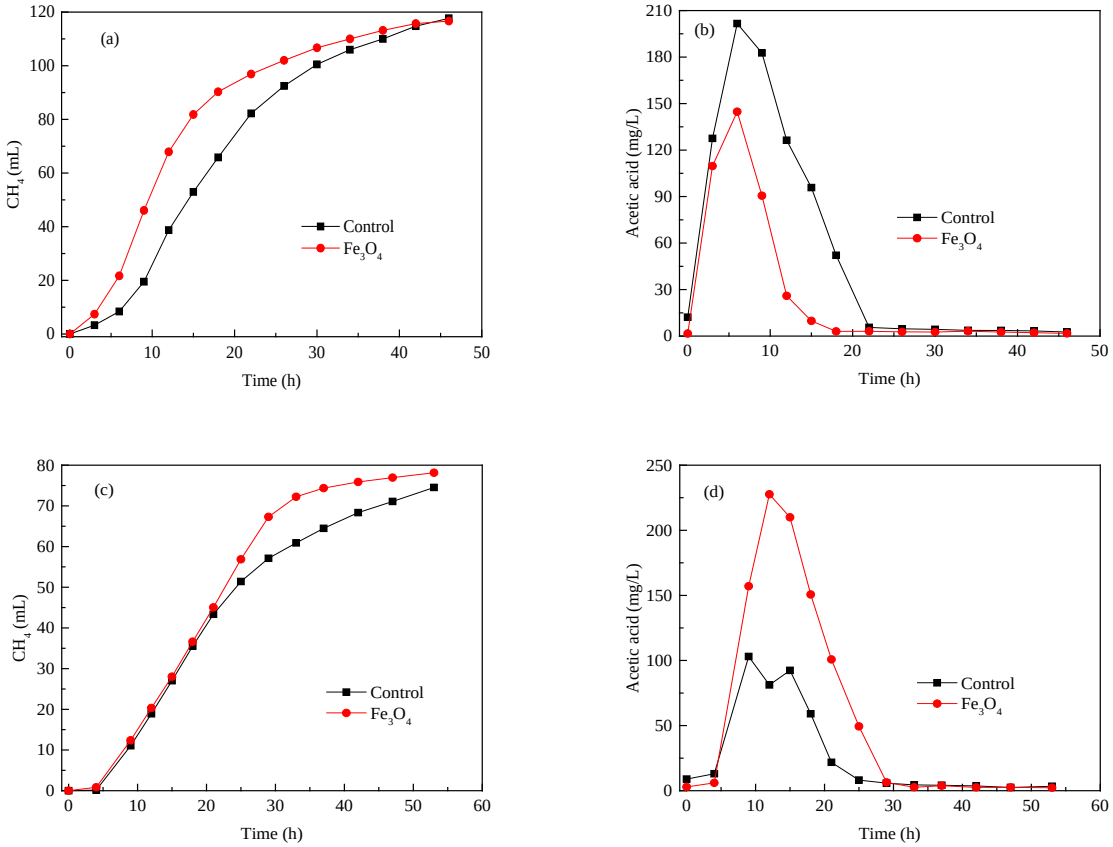

Figure 1. Methane production (a) and acetic acid production (b) using tryptone as carbon source; methane production (c) and acetic acid production (d) using starch as carbon source.

For the anaerobic sludge acclimated with protein-based substrate, with the addition of Fe_3O_4, the lag phase was shortened and the CH_4 production rate was increased, while the addition of Fe_3O_4 had little effect on the total amount of the produced CH_4. These data were well fitted with the modified Gompertz model (R^2 of 0.999 and 0.995 respectively). The obtained ultimate CH_4 yield of the control group and the Fe_3O_4 group was 117.3 mL and 112.5 mL; the lag phase time was 5.0 h

and 2.6 h; the maximum CH_4 production rate, R_{max}, was 5.1 mL/h and 6.9 mL/h, respectively. Therefore, with the addition of Fe_3O_4, the lag phase was shortened by 49.0% and the maximum CH_4 production rate was increased by 34.2%. The VFAs consumption rate, especially acetic acid, was faster by adding Fe_3O_4. On the 15th hour, the acetic acid concentration of the control group and the Fe_3O_4 group was 95.8 mg/L and 9.8 mg/L, respectively.

For the anaerobic sludge acclimated with starch-based substrate, the addition of Fe_3O_4 had little effect on the CH_4 production rate or the lag phase, while the produced acetic acid concentration was increased. During the experiment, the R_{max} of the control group and the Fe_3O_4 group was 2.8 mL/h and 3.3 mL/h, and the lag phase was 5.4 h and 6.4 h, respectively. However, the change of VFAs during the experiment was different, especially the acetic acid. The highest acetic acid concentration of the control group and the Fe_3O_4 group was 103.1 mg/L and 227.6 mg/L, respectively.

Effect of Fe_3O_4 concentrations on methanogenic degradation of tryptone or starch

Fig. 2 shows the result of the concentrations of Fe_3O_4 on anaerobic degradation of tryptone or starch.

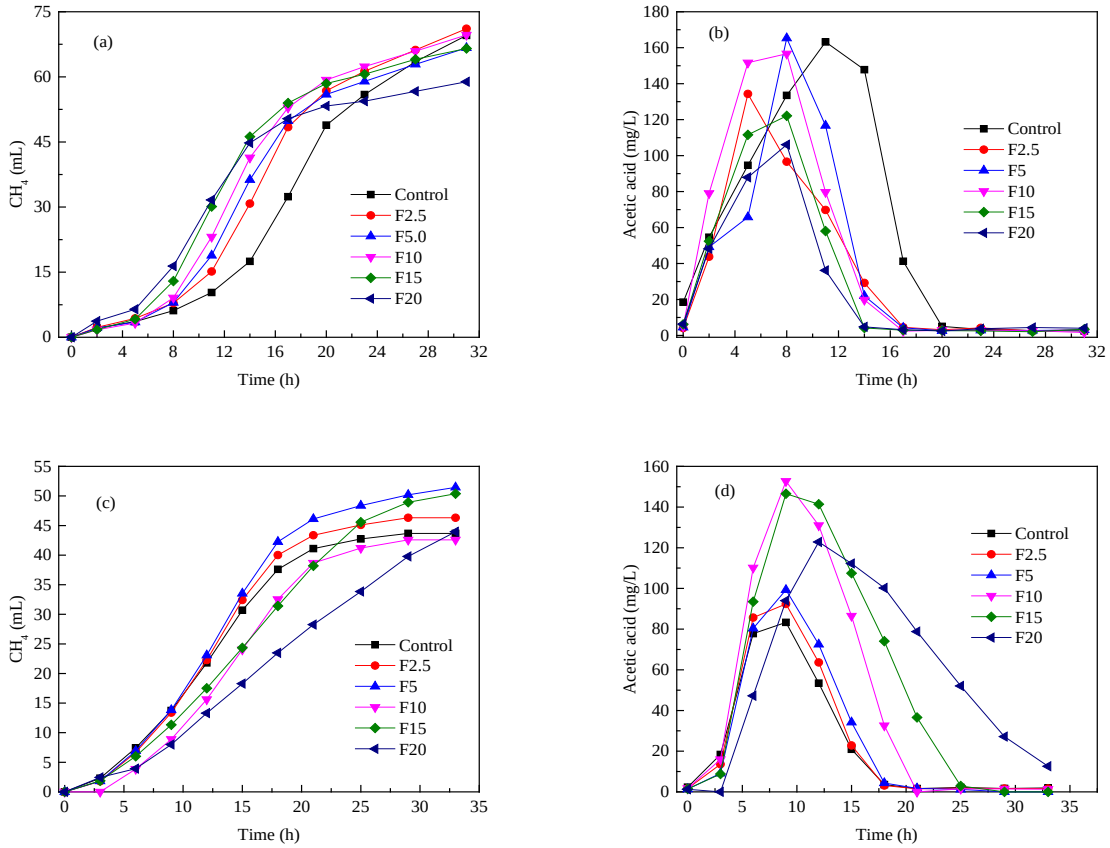

Figure 2. Methane production (a) and acetic acid production (b) with varying Fe_3O_4 concentrations using tryptone as carbon source, and methane production (c) and acetic acid production (d) with varying Fe_3O_4 concentrations using starch as carbon source.

For the anaerobic sludge acclimated with protein-based substrate, with increasing the Fe_3O_4 concentration, the lag phase was shortened. Modified Gompertz model fitting results showed that the lag phase of the control group, F2.5, F5, F10, F15 and F20 was 9.2 h, 7.5 h, 7.0 h, 6.6 h, 5.5 h and 4.3 h, respectively. The maximum CH_4 production rate was 4.3 mL/h, 5.0 mL/h, 5.2 mL/h, 5.5 mL/h, 5.5 mL/h and 4.9 mL/h, respectively. Thus, except F20, the maximum CH_4 production rate increased with the increase of Fe_3O_4 concentration.

For the anaerobic sludge acclimated with starch-based substrate, the methane production rate was not facilitated by the dosage of different Fe_3O_4 concentrations. The lag phase of the control group, F2.5, F5, F10, F15 and F20 was 4.3 h, 4.9 h, 4.9 h, 6.4 h, 4.8 h and 5.0 h, respectively. The R_{max} was 3.0 mL/h, 3.4 mL/h, 3.5 mL/h, 3.0 mL/h, 2.5 mL/h and 1.8 mL/h, respectively. Therefore, various Fe_3O_4 concentrations all led to longer lag phase and only the R_{max} of F2.5 and F5 increased. Moreover, the highest acetic acid concentration of the control, F2.5, F5, F10, F15 and F20 was 88.3 mg/L, 92.4 mg/L, 99.2 mg/L, 152.7 mg/L, 146.5 mg/L, 146.5 mg/L and 122.8 mg/L, respectively. Thus the dosage of Fe_3O_4 increased the maximum acetic acid concentration to different levels.

Effect of Fe_3O_4 on hydrolysis and acidification of tryptone

To further evaluate the effect of Fe_3O_4 on the hydrolysis and acidification of tryptone, 10 mmol/L BES was added to inhibit methanogens. During the hydrolysis and acidification phase, no CH_4 was produced while propionic acid, butyric acid and acetic acid was accumulated gradually. The final VFAs concentration of the control group and the Fe_3O_4 group was 626.2 mg/L and 629.7 mg/L, respectively. The VFAs - COD ratio was 69.0% and 75.1%, respectively. Fig. 3 shows that the control group and the Fe_3O_4 group had similar trend of VFAs production, indicating that Fe_3O_4 might not facilitate the hydrolysis of tryptone. Similar results were obtained by the metabolic end product of hydrolysis and acidification, acetic acid.

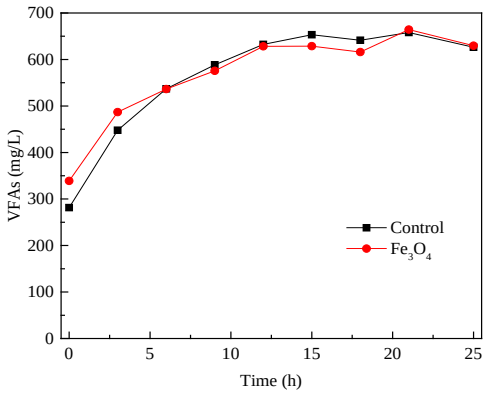

Figure 3. VFA production during hydrolysis and acidification of tryptone.

Effect of Fe_3O_4 on methane producing phase

To examine whether Fe_3O_4 enhances the activities of methanogens without the participation of hydrolysis and acidification, sodium acetate was used as the carbon source instead of tryptone. However, without hydrolysis and acidification, adding Fe_3O_4 seemed to hinder the activities of methanogen (Fig. 4). According to the modified Gompertz model, the lag phase time of the control group and the Fe_3O_4 group was 17.9 h and 19.6 h; the R_{max} was 6.4 mL/h and 1.7 mL/h; the obtained ultimate CH_4 yield was 72.6 mL and 67.9 mL, respectively. So when sodium acetate was used as the carbon source, the R_{max} was decreased by 73.1% and the lag time was delayed by 9.7%.

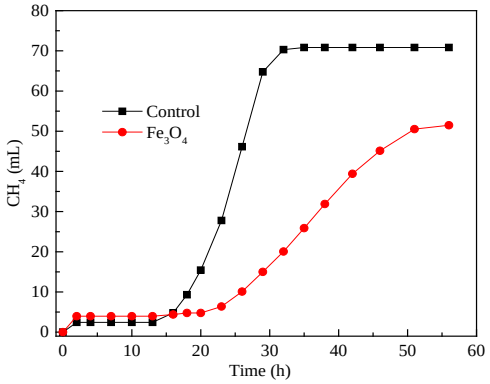

Figure 4. Methane production using NaAc as the organic carbon source.

Community structure analysis

Fig. 5 shows the microbial communities of the anaerobic sludge acclimated with tryptone or starch.

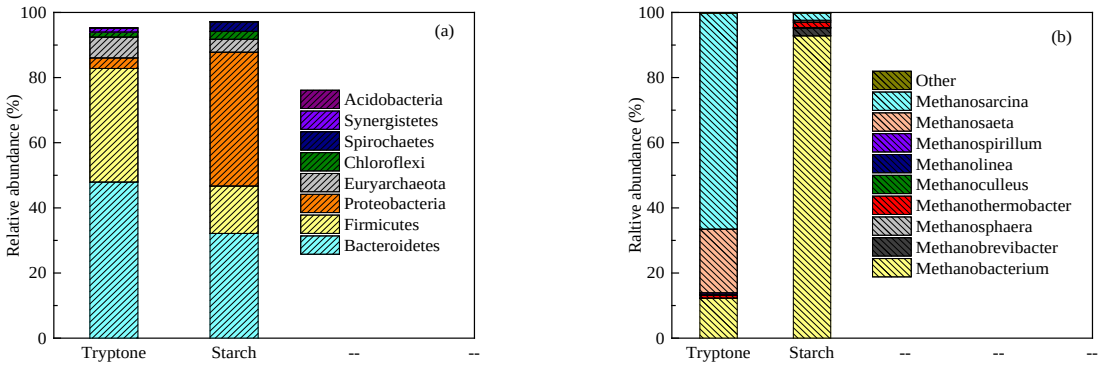

Figure 5. Relative abundance of microbial communities at phylum level of the sludge acclimated with tryptone or starch (a) and archaeal community structure at genus level of the sludge acclimated with tryptone or starch (b).

At the phylum level, *Bacteroidetes* and *Firmicutes* were dominant in the sludge acclimated with tryptone, accounted for 47.9% and 34.9%, respectively. *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were predominant in the sludge acclimated with starch, accounted for 41.1%, 32.2% and 14.57%, respectively. At the genus level, *Methanosarcina*, *Clostridium*, *Syntrophomonas* and *Methanosaeta* were dominant species in the sludge acclimated with tryptone, accounted for 4.1%, 3.4%, 1.9% and 1.2%, respectively. *Aeromonas*, *Azonexus*, *Thauera*, *Acinetobacter*, *Methanobacterium* were predominant in the sludge acclimated with starch, accounted for 15.6%, 6.6%, 4.7%, 3.8% and 3.7%, respectively.

The dominant genus of methanogenic archaea in the sludge acclimated with tryptone was *Methanosarcina*, *Methanosaeta* and *Methanobacterium*, accounted for 66.3%, 19.6% and 12.2%, respectively. In the sludge acclimated with starch, *Methanobacterium* was the most dominant methanogenic archaea, accounted for 92.8%.

DISCUSSION

From the results above, it showed that Fe_3O_4 could accelerate CH_4 production for microorganism acclimated with tryptone. The lag phase was shortened by 18.3% - 53.1%, and the maximum CH_4

production rate was increased by 13.0% - 28.0% with dosing different concentrations of Fe_3O_4. In addition, the acceleration increased with increasing Fe_3O_4 concentrations. In the Fe_3O_4 dosed groups, VFAs reached a peak earlier and then degraded earlier than the control group. Cruz Viggi et al. (2014) also obtained the same tendency when dosing magnetite in anaerobic sludge fed with propionic acid. However, the acceleration only occurred when the interspecies electron transfer between acidogenic bacteria and methanogen was enhanced. The dosage of Fe_3O_4 on hydrolysis and acidification phase or methane producing phase did not improve VFAs production or CH_4 production. Therefore, the conductive property of Fe_3O_4 might be the key factor accelerating DIET for syntrophic CH_4 production (Viggi et al., 2014; Li et al., 2015a) and a high Fe_3O_4 concentration provided a better conductive condition leading to a high acceleration. When sodium acetate was used as the carbon source, interspecies electron transfer was absent. So under this circumstance, the addition of Fe_3O_4 might inhibit the transfer efficiency between methanogens and sodium acetate, leading to the decreased R_{max} and the increased lag phase.

However, when Fe_3O_4 was dosed to the sludge acclimated with starch, the acceleration of CH_4 production did not occur. The lag phase was prolonged by 12.2% - 48.8%. The R_{max} of F2.5 and F5 was increased by 12.0% and 15.0%, respectively. But the R_{max} of other Fe_3O_4 groups, F10, F15 and F20, was decreased by 2.0%, 19.0% and 41.0%, respectively. Moreover, dosing Fe_3O_4 also increased the maximum VFAs accumulation by 7.0% - 62.9%. It indicated that the increased accumulation of VFAs was probably due to the low efficiency of CH_4 production rather than hydrolysis and acidification, according to the prolonged lag phase.

Many carbon sources were used in similar researches to examine the facilitation of conductive materials, such as ethanol (Chen et al., 2014, Rotaru et al., 2014, Liu et al., 2012), glucose (Luo et al., 2015), butyrate (Li et al., 2015a), propionate (Cruz Viggi et al., 2014; Yamada et al., 2015), and benzoate (Zhuang et al., 2015). All showed the acceleration of CH_4 production. However, in our study, when Fe_3O_4 was dosed in the anaerobic sludge acclimated with starch, no such enhancement was observed. Therefore, carbon sources seemed to affect the performance of conductive materials. In the sludge acclimated with trypton, the relative abundance of *Methanosarcina*, *Methanosaeta* and *Methanobacterium* accounted for 66.3%, 19.6% and 12.2%, respectively. *Methanosarcina* and *Methanosaeta* were shown to be able to accept electrons via DIET (Rotaru et al., 2014a; Morita et al., 2011) and these two species occupied over 85.9% of the total archaea. Nevertheless, in the sludge acclimated with starch, *Methanosarcina* and *Methanosaeta* only accounted for 2.8% of the total archaea. On the other hand, *Methanobacterium*, a typical H_2-utlizing methanogen, was the most abundant methanogen in the sludge acclimated with starch, accounting for 92.8%. Whether *Methanobacterium* involved in DIET was still controversial (Morita et al., 2011; Li et al., 2015; Rotaru et al., 2014b). According to results above, *Methanobacterium* might not participate in DIET. Thus the different proportion of methanogens might explain why the sludge acclimated with tryptone was enhanced by dosing Fe_3O_4, while the sludge acclimated with starch was not.

Due to the DIET ability of *Geobacter*, which can metabolize ethanol to acetate, many studies have used co-cultures of *Geobacter* to testify the effect of conductive materials (Chen et al., 2014, Rotaru et al., 2014a, Liu et al., 2012). However, in the sludge fed with tryptone, no *Geobacter* was detected and in the sludge fed with starch, the relative abundance of *Geobacter* accounted for only 0.35%. These results were reasonable because *Geobacter* mainly consumed ethanol to produce acetate but not tryptone or starch. In the sludge fed with tryptone, at the phylum level, *Bacteroidetes* and *Firmicutes* were dominant, accounted for 47.9% and 34.9%, respectively. *Bacteroidetes* and *Firmicutes* might play important roles in protein and starch degradation (Kampmann et al., 2012). In the sludge acclimated with starch, *Proteobacteria* was the most abundant fermentating bacteria, accounting for 41.1%. The next most abundant bacteria was *Bacteroidetes* and *Firmicutes*,

accounting for 32.2% and 14.6%, respectively. Therefore, other bacteria other than *Geobacter* might possibly participate in DIET. Cruz et al. (2014) believed that magnetite particles facilitated DIET between acetogens which oxidize propionate and methanogens to promote propionate consumption and CH_4 production. Li et al. (2015b) dosed single-walled carbon nanotubes in anaerobic digester and enhanced CH_4 production and sucrose decomposition, despite that no *Geobacter* was detected. Zhao et al. (2016) reported that *Syntrophomonas* species was enriched in two carbon felt reactors which were also promoted DIET, and proposed that *Syntrophomonas* species were likely to participate in DIET for sludge decomposition and CH_4 production. In the present study, within the *Firmicutes* phylum of sludge acclimated with tryptone, 15.0% of the species were assigned to the genus *Syntrophomonas*. However, the proportion was only 1.0% in the sludge acclimated with starch. Therefore, it could be further confirmed that some species other than *Geobacter* such as *Syntrophomonas* could also transfer electrons via DIET. Microbial communities of anaerobic sludge could be shaped by different carbon sources, showing different responses when conductive materials were dosed.

CONCLUSIONS

The dosage of Fe_3O_4 accelerated methane production for microorganisms acclimated with protein-based substrate and this acceleration only occurred when the interspecies electron transfer between acidogenic bacteria and methanogen existed. The conductive property of Fe_3O_4 might be the reason for the acceleration of DIET for syntrophic CH_4 production. *Methanosarcina* and *Methanosaeta* were dominant in the sludge fed with tryptone, while *Methanobacterium* was dominant in the sludge fed with starch. Organic carbon affected the acclimated microbial communities, leading to different performance when dosing conductive materials.

ACKNOWLEDGEMENT

This research was supported by Shenzhen Science and Technology Plan - Fundamental Research (JCYJ20150331151358156).

REFERENCES

APHA, 1995 Standard methods for the examination of water and wastewater. *American Public Health Association*, Washington DC.

Chen S, Rotaru A E, Liu F, Philips J, Woodard T L, Nevin K P, Lovley D R. 2014 Carbon cloth stimulates direct interspecies electron transfer in syntrophic co-cultures. *Bioresource Technology* **173**, 82-86.

Cruz Viggi C, 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. *Environmental Science & Technology* **48**, 7536-7543.

Guo F, Zhang T. 2012 Profiling bulking and foaming bacteria in activated sludge by high throughput sequencing. *Water Research* **46**(8), 2772-2782.

Kampmann K, Ratering S, Kramer I, Schmidt M, Zerr W, Schnell S. 2012 Unexpected stability of *Bacteroidetes* and *Firmicutes* communities in laboratory biogas reactors fed with different defined substrates. *Applied and Environmental Microbiology* **78**, 2106-2119.

Li H, Chang J, Liu P, Fu L, Ding D, Lu Y. 2015a Direct interspecies electron transfer accelerates syntrophic oxidation of butyrate in paddy soil enrichments. *Environmental Microbiology* **17**, 1533-1547.

Li L L, Tong Z H, Fang C Y, Chu J, Yu H Q. 2015b Response of anaerobic granular sludge to single-wall carbon nanotube exposure. *Water Research* **70**, 1-8.

Liu F, Rotaru A E, Shrestha P M, Malvankar N S, Nevin K P, Lovley D R. 2012 Promoting direct interspecies electron transfer with activated carbon. *Energy & Environmental Science* **5**, 8982.

Lovley D R. 2011 Reach out and touch someone: potential impact of DIET (direct interspecies

energy transfer) on anaerobic biogeochemistry, bioremediation, and bioenergy. *Reviews in Environmental Science and Bio/Technology* **10**(2), 101-105.

Luo C, Lu F, Shao L, He P. 2015 Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. *Water Research* **68**, 710-718.

Morita M, Malvankar N S, Franks A E, Summers Z M, Giloteaux L, Rotaru A E, Rotaru C, Lovley D R. 2011 Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates. *MBio* **2**(4), e00159-11.

Rotaru A E, Shrestha P M, Liu F, Markovaite B, Chen S, Nevin K P, Lovley D R. 2014a Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*. *Applied and Environmental Microbiology* **80**, 4599-4605.

Rotaru A-E, Shrestha P M, Liu F, Shrestha M, Shrestha D, Embree M, Zengler K, Wardman C, Nevin K P, Lovley D R. 2014b A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. *Energy & Environmental Science* **7**, 408-415.

Shrestha P M, Malvankar N S, Werner J J, Franks A E, Elena-Rotaru A, Shrestha M, Liu F, Nevin K P, Angenent LT, Lovley D R. 2014 Correlation between microbial community and granule conductivity in anaerobic bioreactors for brewery wastewater treatment. *Bioresource Technology* **174**, 306-310.

Yamada C, Kato S, Ueno Y, Ishii M, Igarashi Y. 2015 Conductive iron oxides accelerate thermophilic methanogenesis from acetate and propionate. *Journal of Bioscience and Bioengineering* **119**, 678-682.

Zhao Z, Zhang Y, Quan X, Zhao H. 2016 Evaluation on direct interspecies electron transfer in anaerobic sludge digestion of microbial electrolysis cell. *Bioresource Technology* **200**, 235-244.

Zhu D, Wang J, Chen T H, Tan J, Yao D F. 2015 Comparison of hematite-facilitated anaerobic digestion of acetate and beef extract. *Environmental Technology* **36**, 2295-2299.

Zhuang L, Tang J, Wang Y, Hu M, Zhou S. 2015 Conductive iron oxide minerals accelerate syntrophic cooperation in methanogenic benzoate degradation. *Journal of Hazardous Materials* **293**, 37-45.

Zwietering M, Jongenburger I, Rombouts F, Van't Riet K. 1990 Modeling of the bacterial growth curve. *Applied and Environmental Microbiology* **56**(6), 1875-1881.