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RESEARCH ARTICLE

Enhanced methane recovery and exoelectrogen-methanogen evolution from low-strength wastewater in an up-flow biofilm reactor with conductive granular graphite fillers

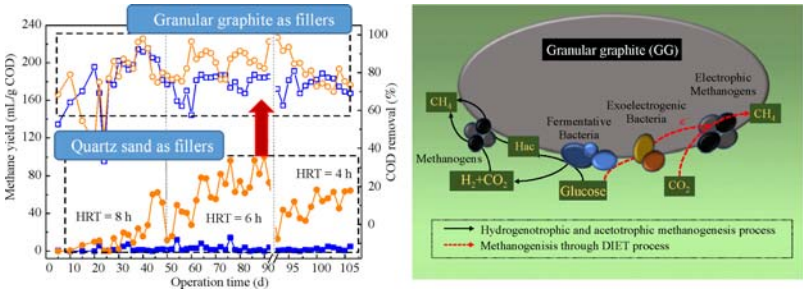
Zechong Guo[1], Lei Gao[1], Ling Wang[1], Wenzong Liu (✉)[2], Aijie Wang[1,2]

1 State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (SKLUWRE, HIT), Harbin 150001, China
2 Key Laboratory of Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*

HIGHLIGHTS

- Methane yield increased 22 times from low-strength wastewater by applying conductive fillers.
- Conductive fillers accelerated the start-up stage of anaerobic biofilm reactor.
- Conductive fillers altered methanogens structure.

GRAPHIC ABSTRACT



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ABSTRACT

Methane production from low-strength wastewater (LSWW) is generally difficult because of the low metabolism rate of methanogens. Here, an up-flow biofilm reactor equipped with conductive granular graphite (GG) as fillers was developed to enhance direct interspecies electron transfer (DIET) between syntrophic electroactive bacteria and methanogens to stimulate methanogenesis process. Compared to quartz sand fillers, using conductive fillers significantly enhanced methane production and accelerated the start-up stage of biofilm reactor. At HRT of 6 h, the average methane production rate and methane yield of reactor with GG were 0.106 $m^3/(m^3 \cdot d)$ and 74.5 L/kg COD, which increased by 34.3 times and 22.4 times respectively compared with the reactor with common quartz sand fillers. The microbial community analysis revealed that methanogens structure was significantly altered and the archaea that are involved in DIET (such as *Methanobacterium*) were enriched in GG filler. The beneficial effects of conductive fillers on methane production implied a practical strategy for efficient methane recovery from LSWW.

1 Introduction

The low-strength wastewater (LSWW) with low organic and nutrient concentration, is discharged from pretreatment processes of enterprises or collected from communities in the form of domestic wastewater. Traditionally, aerobic processes are employed to mineralize the organic matters in LSWW to satisfy the strict discharge standard (Chan et al., 2009). But the serious energy shortage and increasingly strict energy-saving policies pose great challenges for the sustainability of current process due to its high-energy-consumption and high-carbon-release (Gu et al., 2016; Wang et al., 2016). Therefore, some leading energy-saving processes for the treatment of LSWW has been proposed (Verstraete et al., 2009), which introduce anaerobic processes to recover energy (such as biogas and electricity)

✉ Corresponding author
E-mail: wzliu@rcees.ac.cn

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from LSWW and then provide it to other sections to achieve the energy-neutral of overall treatment process (McCarty et al., 2011; Batstone and Virdis, 2014).

Despite its attractive prospects, biogas-recovery from LSWW is still limited by the low methane-producing efficiency of anaerobic systems under the condition of insufficient nutrient (Brito et al., 1997; Lettinga et al., 2001). Although some novel anaerobic systems (such as anaerobic membrane reactors) are efficient in producing methane with LSWW (Martinez-Sosa et al., 2011; Li et al., 2017a), they are not the preferred technologies for energy recovery considering of the high energy cost of themselves (Smith et al., 2014). One important reason for the unsatisfied performance of anaerobic systems is that methanogens grow very slowly under adverse conditions (such as low concentration of substrates or low temperature) (Speece, 1996) and thus they are generally difficult to obtain sufficient biomass or maintain a high metabolic activity (Sangeetha et al., 2017). Therefore, it is of great significance to develop a feasible strategy to simulate the growth and metabolism of methanogens and upgrade the current anaerobic technologies for efficient methane production from LSWW.

Recently, a new understanding that the electron transfer efficiency plays a key role in anaerobic digestion process has been put forward (Summers et al., 2010; Lovley, 2011). It was disclosed that direct interspecies electron transfer (DIET) in syntrophic metabolism can be enhanced by introducing electrically conductive materials (such as carbon and metallic materials) in anaerobic bioreactors (Liu et al., 2012; Chen et al., 2014a; 2014b; Li et al., 2017b; Zhao et al., 2017), and significantly accelerate the methanogenesis process and methanogens growth (Summers et al., 2010; Morita et al., 2011;Storck et al., 2016; Wang et al., 2017). These findings provide a potential approach to upgrading anaerobic technology for efficient methane recovery from LSWW. It is worth noting that the previous research results were mainly obtained in bioreactors containing granular or flocculent sludge, but little attention was paid on anaerobic biofilm reactors. The effect of conductive materials on the evolution of methanogens in anaerobic biofilm has not been thoroughly investigated. The possibility of building an upgraded anaerobic biofilm reactor for efficient methane recovery from LSWW has not been explored.

Therefore, in this study, electric conductive granular graphite was employed, instead of conventional nonconductive fillers, in an up-flow biofilm reactor for low-strength artificial wastewater (COD 400 mg/L) treatment at ambient temperature. The methane production and organic matter removal performance were examined under different hydraulic retention time (HRT) conditions. The influence of applying conductive fillers on exoelectrogen-methanogen microbial community structure shift was discussed.

2 Materials and methods

2.1 Up-flow biofilm reactor configuration

Two up-flow biofilm reactors were constructed with different fillers (Fig. 1). Each reactor was made of plexiglass cylinder (inner diameter of 8 cm in diameter and a height of 25 cm), with a liquid volume of 1 L. One reactor was equipped conductive granular graphite fillers (noted as GG) (Beihai Carbon Co., Ltd. China), and the other one used nonconductive quartz sand as fillers (noted

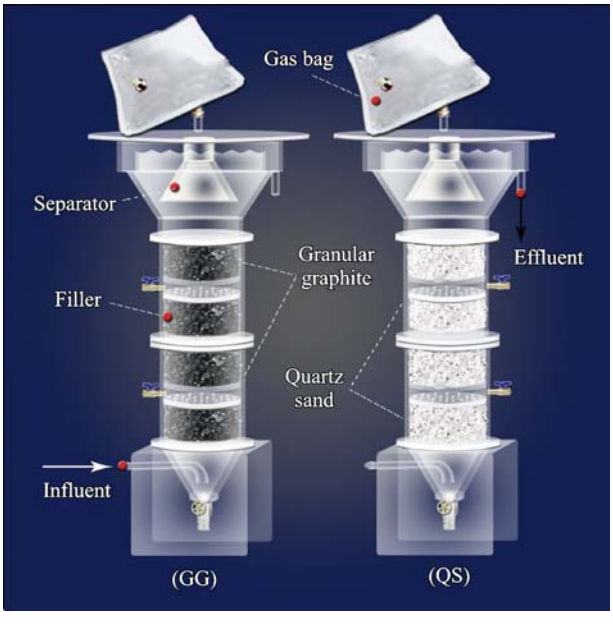

Fig. 1 Schematic diagram of reactor configuration

as QS) (Heshun Mining Co., Ltd, China), the total volume of fillers was 0.6 L in both reactors. Before used, granular graphite and quartz sand were screened out to have same particle sizes (3−5 mm) to make sure two reactors have similar hydrodynamic properties (seen in supplementary material S1). The granular graphite was pretreated according to previous literature (Cui et al., 2016b).

2.2 Startup and operation conditions

Both reactors were inoculated with effluent from a long-term operated microbial electrolysis cell and fed with low-strength artificial wastewater, which contained 400 mg/L glucose, 50 mmol phosphate buffer solution (PBS) and trace elements (Sangeetha et al., 2017). The hydraulic retention time (HRT) of each reactor was decreased from 8 to 4 h, and the entire experiment lasted for more than 120 days. All experiments were carried out at ambient temperature (25±3°C).

2.3 Analytical and calculation methods

Liquid samples that collected from reactor were immediately filtered through 0.45 μm filters before analysis (Cui et al., 2016a). Chemical oxygen demand (COD) was analyzed according to the standard methods (Potassium Dichromate Method). Gas composition (methane, hydrogen and carbon dioxide) was analyzed by a gas chromatograph (Agilent 7890, USA). All gas volume data were calculated at standard condition according to ideal gas law.

COD balance analysis was conducted for QS and GG with the operational data under the HRT of 6 h, the proportion of organic matters in influent (calculated as COD) converted to gaseous and dissolved methane, residual soluble organics in effluent, suspended organic matters in effluent (including detached biomass and intercellular polymers) and other unknown lost, were calculated. The calculation formula were list in supplementary material S2.

2.4 Biofilm sampling and Illumina sequencing

After 120 days operation, GG and QS were taken out from reactor and washed with sterilized water 8–10 times to fully detach the biofilm from carriers. All liquid was mixed together and concentrated by centrifugation (8000 rpm). The supernatant was discarded and the precipitates were saved under –20°C for subsequent analysis. The extraction of total genomic DNA of biofilm samples, and bacterial and archaeal sequencing analysis were conducted according to previous literatures (Cai et al., 2016; Liu et al., 2016), which were described in details in supplementary material S3.

3 Results and discussion

3.1 Methane production and organic matter removal

The methane production of QS maintained a low level during the overall operation period (Fig. 2). Under the HRT of 8 h (organic loading of 1.2 kg COD/(m^3·d)), the average methane production rate, methane content and methane yield of QS were 0.002 m^3/(m^3·d), 4.3% and 1.81 L/kg COD, respectively. When HRT was shortened to 6 h, an increased organic load of 1.6 kg COD/(m^3·d) resulted in a slight increase in methane production rate, methane content and methane yield, which increased to 0.004 m^3/(m^3·d), 8.3% and 3.18 L/kg COD respectively. But further shortening HRT to 4 h led to a reduced methane production to 0.003 m^3/(m^3·d) and 1.95 L/kg COD, because of the insufficient time for methanogenesis at such low HRT.

A substantial enhancement in methane production was achieved in GG compared with QS. The methane production began to rise rapidly and reached a relative stable stage after 40 days. When HRT was shortened to 6 h, methane production continuously promoted due to the increased organic load. The average methane production rate and methane yield of GG reached 0.106 m^3/(m^3·d) and 74.5 L/kg COD, which increased by 34.3 times and 22.4 times, respectively, compared with QS. Further shortening HRT to 4 h, average methane production rate and methane yield slightly decreased to 0.091 m^3/(m^3·d) and 50.8 L/kg COD, respectively. Meanwhile, COD removal efficiency of GG was relatively stable, and the average COD removals under HRT 8 h, 6 h and 4 h were 84.1%, 85.5% and 80.4% respectively, which were all higher than those in QS (83.6%, 75.8% and 73.5%) (Fig. 2b).

COD balance analysis was conducted in QS and GG to disclose the conversion process of organic matters in two systems (Fig. 3). In QS, only about 3.5% of organic matters in influent were converted to methane, and most part were dissolved in liquid phase (2.9%). About 29% of organic matters remained in effluent as soluble COD, and 47% were transferred into suspended matters in effluent, which mainly included the biomass detached from biofilm and intercellular poly-compounds, and the residual parts account for some unknown lost. It has been reported that considerable proportion of carbohydrate (such as glucose) in influent would first be accumulated as polymers (such as trehalose) in biomass before being utilized for growth or metabolism (Shimada et al., 2007). In this study, most organic matters in influent were actually removed by biosorption instead of complete anaerobic digestion due to a short HRT (6 h).

The situation in GG was substantially improved, the soluble and suspended organics in effluent decreased to 14% and 36% respectively, and the proportion of organic substrate converted to methane gas was promoted to 34%,

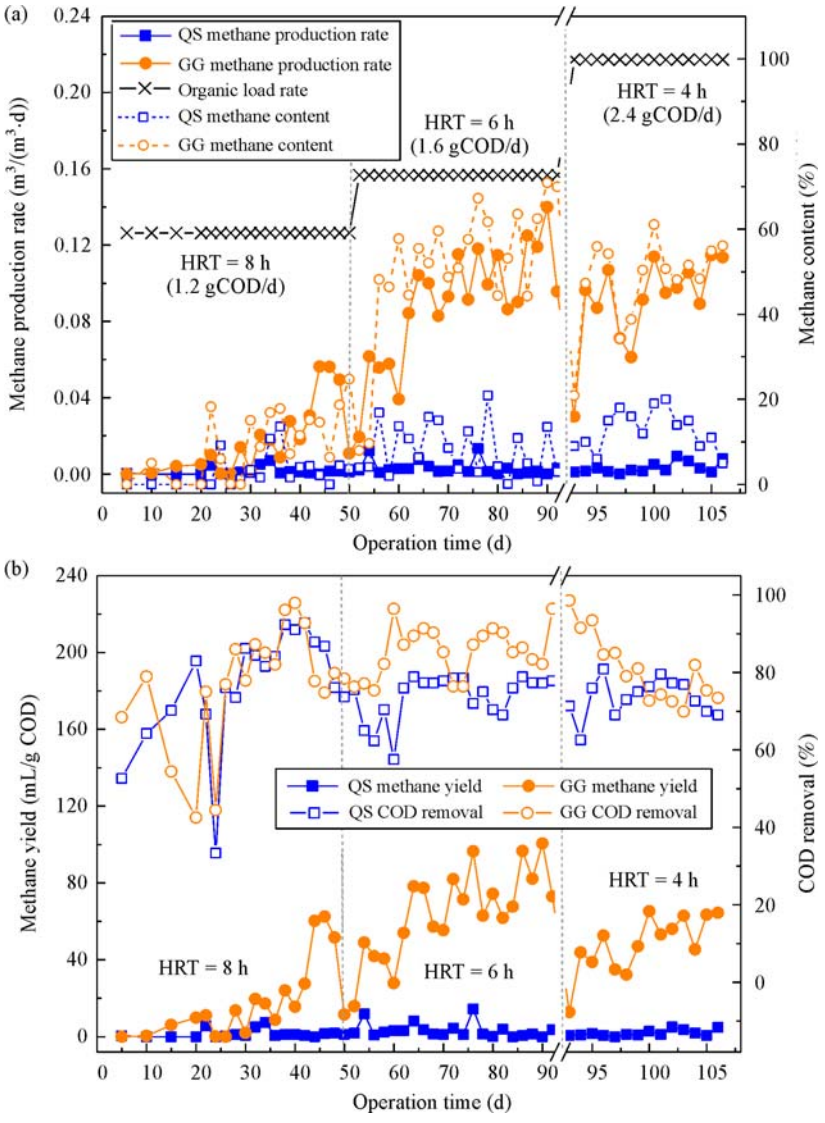

Fig. 2 Organic load and methane production rate (a); COD removal and methane yield (b) in QS and GG under different HRT conditions

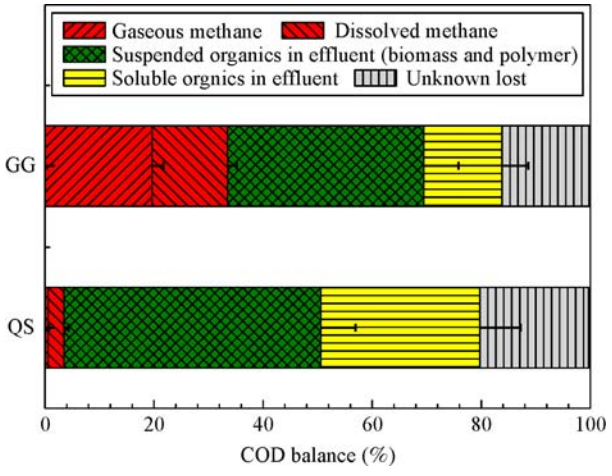

Fig. 3 COD balance analysis in QS and GG

including 20% of methane gas and 14% of dissolved methane. It is clear that using granular graphite as fillers in anaerobic biofilm reactor obviously accelerated the conversion process of organic substrates in LSWW to methane.

Similar results about the promising effect of conductive materials on organic removal and methane production were previously reported in some anaerobic systems with aggregated or flocculent sludge. For example, Zhao et.al (Zhao et al., 2015) found that the methane production of UASB reactors increased 30-45% by adding conductive carbon materials (graphite, biochar and carbon cloth). Luo et.al (Luo et al., 2015) reported an 86.6% increase in methane production rate of an anaerobic digester with granular sludge by adding biostable biochar. Liu et. al. reported that the conductive material granular activated carbon promote DIET and thus significantly enhanced methane production in a methanogenic digester (Liu et al., 2012). The results of this study further proved that the promoting effect of conductive materials (such as granular graphite) also exists in biofilm-based anaerobic reactors, and may be even more intensive (the methane production rate of the upgraded biofilm reactor in this study increased as high as 34 times). That may be because that biofilm reactors are less influenced by inoculum, but more effected by the carrier property.

Considering the similar hydrodynamic properties and operational conditions between QS and GG, the significant enhancement in methane production in GG most likely caused by the changes of microbial community and metabolism function. The conductive granular graphite may have strong screening effect on the enrichment and colonization of functional microbial populations (especially methanogens and the syntrophic bacteria) during the formation of active biofilm, and thus led to a substantial improvement in methane production. Therefore, analyzing microbial community structure from different fillers and their synergistic effect would be benefit for better understanding the mechanism and bring this technology closer to practical application.

3.2 Overall microbial community structures

The rarefaction curves, OUT overlaps and α-diversity indexes of QS and GG revealed an overall difference between the microbial community structures of these two systems. The rarefaction curves of bacteria and archaea at 3% distance thresholds were shown in Fig. 4a. The rarefaction curves of QS and GG were very close in bacterial sequencing, but they showed an obvious difference in archaeal sequencing. More OTUs were obtained from QS than GG at the same sequences in archaeal sequencing, which implied a relatively higher archaeal diversity of QS than GG.

The total classified OTUs of bacteria group in QS and GG was 570 and 293 OTUs (51.4%) were shared by them,

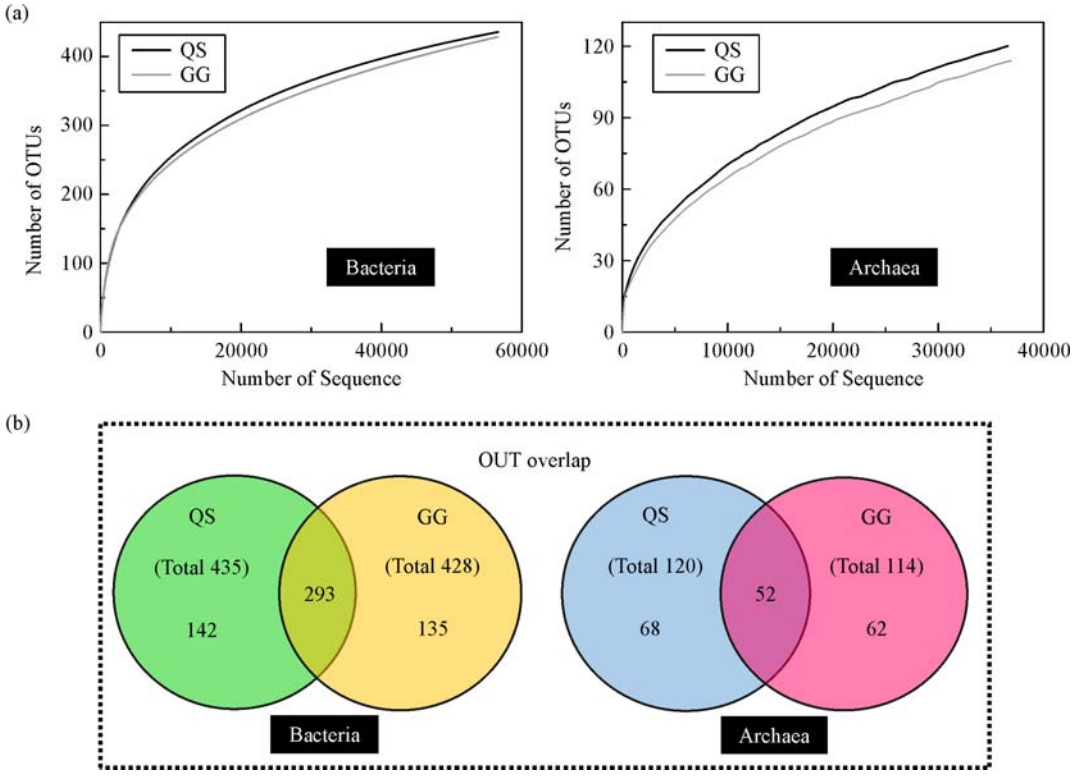

Fig. 4 Rarefaction curves of bacterial and archaeal communities in QS and GG (a); OTU overlaps bacterial communities and archaeal communities between QS and GG (b).

as shown in Fig. 4b. Although the composition of thus core bacterial communities from different fillers were quite similar, it was quite different in archaea group, which showed that total OTUs were 182 and only 52 (28.51%) were shared in two reactors.

The abundance-based coverage estimator (ACE), Shannon index and Chao1 index of bacterial community in GG were relatively higher than those of QS, while the Simpson index was relatively lower, which indicated a higher species richness of bacterial community in GG compared with QS, as shown in Table 1. The opposite trend was found in archaea group. The ACE, Chao1 index of GG were lower but Simpson index was relatively higher, which implied the high archaea diversity of QS than GG.

All evidences showed that applying conductive granular graphite as fillers induced an alteration in microbial community structure (especially the archaeal structure) of biofilm in GG, and the decreased archaeal species diversity implied an enrichment of functional methanogens.

3.3 Potential dominant genera contributing to DIET methane production

Methane production in anaerobic biofilm reactors relays on the coexist and cooperation between various bacterial and archaeal populations. In bacterial community, *Lactococcus*, which produces lactate from glucose (Yang et al., 2016), was the most abundant genus in both groups, which were 52.2% and 38.1% in QS and GG, respectively (Fig. 5a). Fermentation bacteria, *Alkaliflexus*, *Sphaerochaeta*, *Propionvibrio*, which are capable of producing acid and hydrogen (Zhilina et al., 2004;Miyazaki et al., 2014), were also dominant in both groups with similar relative abundances.

Traditionally, it was believed that the electrons in syntrophic methanogenesis systems were transferred through electron shuttles (i.e. hydrogen or formate) (Shen et al., 2016). But recently, conductive materials are reported to be able to induce the formation of shuttle-free DIET between electroactive bacteria and electrotrphic methanogens (Rotaru et al., 2014a). In this study, the abundances of *Geobacter*, which was recognized as an electron donating bacteria in DIET (Summers et al., 2010; Rotaru et al., 2014b), was very low in both systems, but there were some other potential electroactive bacteria, such as *Desulfovibrio*, *Desulfuromonas*, *Anaeroarcus*, and *Desulfibacter* (Fig. 5a), which might be capable of transferring electrons to methanogens (Finster et al., 1997; Strömpl et al., 1999; Sass et al., 2009). Although the relative abundances of potential electroactive bacteria were not significantly increased in GG, the existence of conductive fillers built an access for electron transfer from these bacteria to electron-accepting methanogens, which led a noticeable shift in archaeal community structure (Fig. 5b).

The most abundant archaeal genus in QS was *Methanobrevibacter*, which accounted for 36.4% of total archaea, followed by *Methanocorpusculum* (28.2%), *Methanobacterium* (20.8%) and *Methanomassiliicoccus* (7.3%) (Fig. 5b). In the reactor GG, the abundances of archaeal genera *Methanobrevibacter*, *Methanocorpusculum*, *Methanomassiliicoccus* were decreased to 17.8%, 8.8% and 1.8% respectively, while the abundance of *Methanobacterium* significantly increased to 66.1%, which became the most dominant archaeal genus. It has been proved that *Methanobacterium* was capable of accepting electrons from solid electrodes or other extracellular respiratory bacteria (Cheng et al., 2009; Lin et al., 2017). Therefore, the selective enrichment of *Methanobacterium* in GG proposes a possibility that DIET was performing in methanogenesis process in bioreactor with conductive fillers.

3.4 Mechanism and Prospective

The microbial community shift and potential DIET-related genera enrichment in GG give a clue for the mechanism of enhanced methane production. In QS, substrates were converted to hydrogen and acetate by fermentative bacteria, and then consumed by hydrogenotrophic and acetoclastic methanogens to produce methane. In GG, in contrast, besides acetoclastic methanogenesis and hydrogenotrophic methanogenesis, a special DIET-based syntrophic partnership was built between exoelectrogens and electrotrophic methanogens (Fig. 6). Syntrophic partners attached to the surface of granular graphite and utilized them as conduits for electron exchange. This shuttle-free electron transfer process allows methane production thermodynamically and metabolically more efficient and led to a rapid conversion of LSWW to methane (Cheng and Call, 2016; Barua and Dhar, 2017).

Generally, a long HRT (more than 24 h) is necessary for sufficient anaerobic methane recovery (Escudié et al., 2011). Applying conductive fillers, however, accelerated the methanogenesis process, which was recognized as the rate-limiting of anaerobic digestion, and then effectively

Table 1 Alpha diversity indexes of microbial communities

	Group	OTU number	Shannon index	ACE index	Chao1 index	Simpson index
Bacteria	QS	435	2.509	535.029	511.514	0.288
	GG	428	2.802	563.453	562.200	0.186
Archaea	QS	120	2.017	212.261	163.000	0.197
	GG	114	1.524	240.965	161.714	0.397

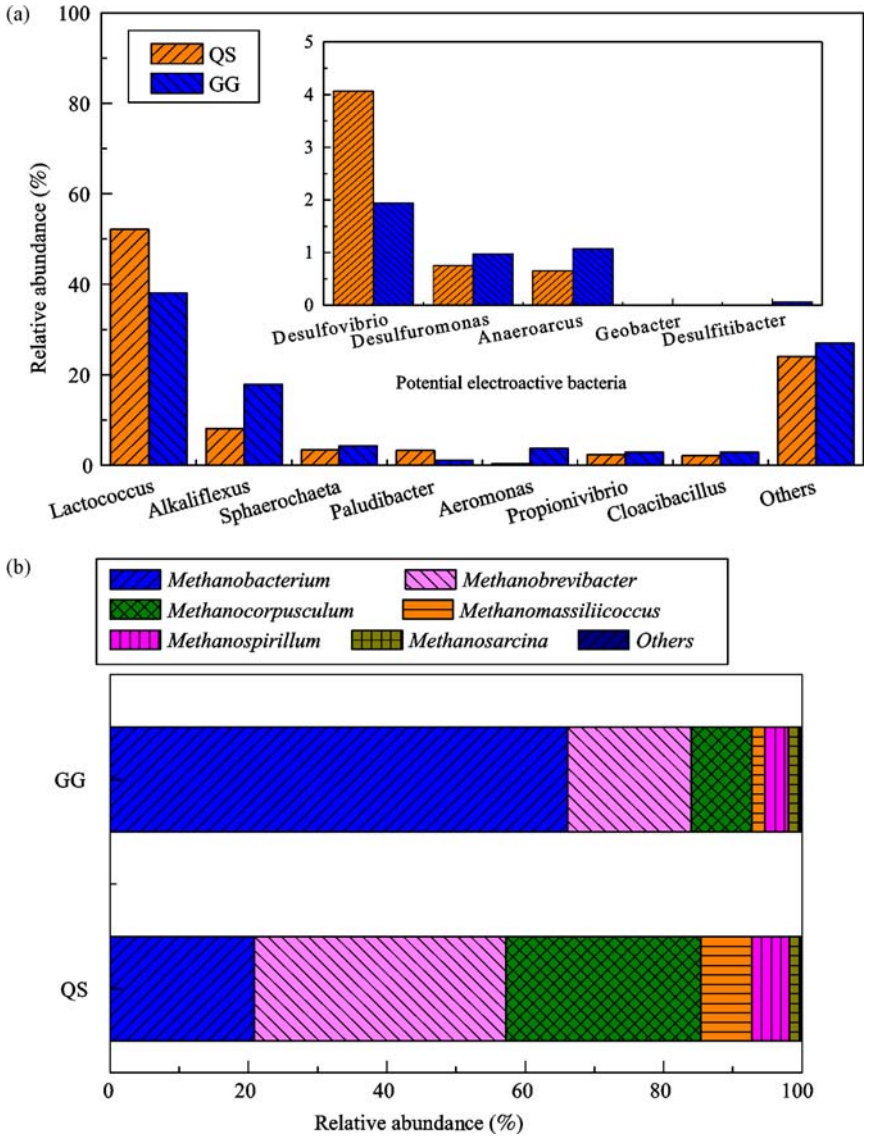

Fig. 5 Bacterial community structure at genus level and potential electroactive bacteria abundance (a); Archaeal community structure at genus level (b) in QS and GG

recovered methane from LSWW under a very short HRT (6 h). From the view of future application, the short HRT is economically favorable for LSWW treatment with anaerobic processes.

The long start-up period seriously limits the widely application of anaerobic biofilm systems, which often takes months to develop an active biofilms in these systems (Escudié et al., 2011). For example, in this study, methanogenesis was hardly observed in the first 40 days and still maintained a low level after over 120 days operation in QS reactor. Fortunately, the noticeably shortened start-up period in system with conductive fillers provides a potential solution for this key problem and increases the economical competitiveness of the anaerobic processes.

In summary, applying conductive fillers is a simple yet effective strategy to promote methane yield, shorten HRT and reduce start-up period of anaerobic biofilm system, which provides a guidance for building biofilm reactors for LSWW treatment or upgrading the existing systems for improving methane recovery.

4 Conclusions

Conductive fillers granular graphite was used to replace common nonconductive fillers in an anaerobic biofilm reactor. Archaeal community structure obvious altered and the enriched genera were capable of direct interspecies electron transfer (such as *Methanobacterium*). Methane was successfully recovered from LSWW at ambient temperature and the start-up stage was apparently

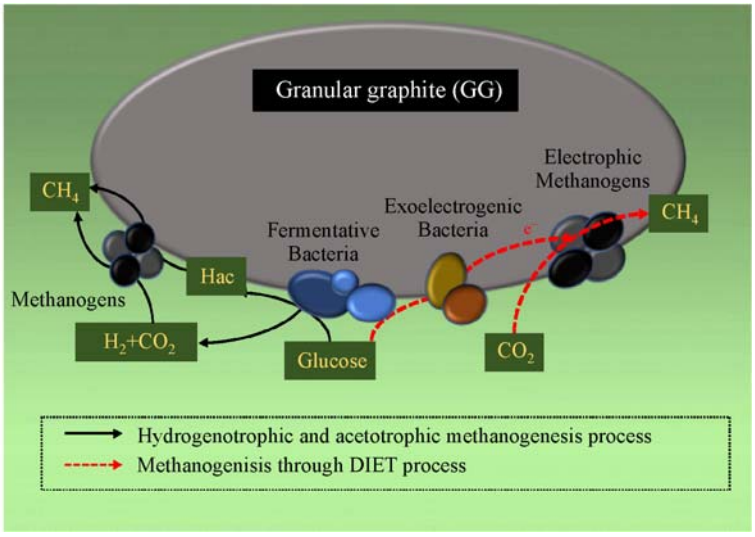

Fig. 6 Schematic diagram of methanogenesis pathways in GG

accelerated. These results have strong practical significances for the application of anaerobic biofilm systems in LSWW treatment.

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References

Barua S, Dhar B R (2017). Advances towards understanding and engineering direct interspecies electron transfer in anaerobic digestion. Bioresource Technology, 244(Pt 1): 698–707

Batstone D J, Virdis B (2014). The role of anaerobic digestion in the emerging energy economy. Current Opinion in Biotechnology, 27: 142–149

Brito A G, Rodrigues A C, Melo L F (1997). Granulation during the start-up of a UASB reactor used in the treatment of low strength wastewaters. Biotechnology Letters, 19(4): 363–368

Cai W W, Liu W Z, Yang C X, Wang L, Liang B, Thangavel S, Guo Z C, Wang A J (2016). Biocathodic methanogenic community in an integrated anaerobic digestion and microbial electrolysis system for enhancement of methane production from waste sludge. ACS Sustainable Chemistry & Engineering, 4(9): 4913–4921

Chan Y J, Chong M F, Law C L, Hassell D G (2009). A review on anaerobic-aerobic treatment of industrial and municipal wastewater. Chemical Engineering Journal, 155(1–2): 1–18

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

Chen S, Rotaru A E, Shrestha P M, Malvankar N S, Liu F, Fan W, Nevin K P, Lovley D R (2014b). Promoting interspecies electron transfer with biochar. Scientific Reports, 4(1): 5019

Cheng Q, Call D F (2016). Hardwiring microbes via direct interspecies electron transfer: Mechanisms and applications. Environmental Science. Processes & Impacts, 18(8): 968–980

Cheng S, Xing D, Call D F, Logan B E (2009). Direct biological conversion of electrical current into methane by electromethanogenesis. Environmental Science & Technology, 43(10): 3953–3958

Cui M H, Cui D, Gao L, Cheng H Y, Wang A J (2016a). Efficient azo dye decolorization in a continuous stirred tank reactor (CSTR) with built-in bioelectrochemical system. Bioresource Technology, 218: 1307–1311

Cui M H, Cui D, Gao L, Wang A J, Cheng H Y (2016b). Azo dye decolorization in an up-flow bioelectrochemical reactor with domestic wastewater as a cost-effective yet highly efficient electron donor source. Water Research, 105: 520–526

Escudi R, Cresson R, Delgens J P, Bernet N (2011). Control of start-up and operation of anaerobic biofilm reactors: An overview of 15 years of research. Water Research, 45(1): 1–10

Finster K, Coates J D, Liesack W, Pfennig N (1997). *Desulfuromonas thiophila* sp. nov., a new obligately sulfur-reducing bacterium from anoxic freshwater sediment. International Journal of Systematic Bacteriology, 47(3): 754–758

Gu Y, Dong Y, Wang H, Keller A, Xu J, Chiramba T, Li F (2016). Quantification of the water, energy and carbon footprints of wastewater treatment plants in China considering a water–energy nexus perspective. Ecological Indicators, 60: 402–409

Lettinga G, Rebac S, Zeeman G (2001). Challenge of psychrophilic anaerobic wastewater treatment. Trends in Biotechnology, 19(9): 363–370

Lin R, Cheng J, Zhang J, Zhou J, Cen K, Murphy J D (2017). Boosting biomethane yield and production rate with graphene: The potential of direct interspecies electron transfer in anaerobic digestion. Bioresource Technology, 239: 345–352

Li N, He L, Lu Y Z, Zeng R, Sheng G P (2017a). Robust performance of a novel anaerobic biofilm membrane bioreactor with mesh filter and carbon fiber (ABMBR) for low to high strength wastewater treatment. The Chemical Engineering Journal,313: 56–64

Li Y, Zhang Y, Yang Y, Quan X, Zhao Z (2017b). Potentially direct interspecies electron transfer of methanogenesis for syntrophic metabolism under sulfate reducing conditions with stainless steel. Bioresource Technology, 234: 303–309

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(10): 8982

Liu W, He Z, Yang C, Zhou A, Guo Z, Liang B, Varrone C, Wang A J (2016). Microbial network for waste activated sludge cascade utilization in an integrated system of microbial electrolysis and anaerobic fermentation. Biotechnology for Biofuels, 9(1): 83

Lovley D R (2011). Live wires: Direct extracellular electron exchange for bioenergy and the bioremediation of energy-related contamination. Energy & Environmental Science, 4(12): 4896

Luo C, L 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

Martinez-Sosa D, Helmreich B, Netter T, Paris S, Bischof F, Horn H (2011). Anaerobic submerged membrane bioreactor (AnSMBR) for municipal wastewater treatment under mesophilic and psychrophilic temperature conditions. Bioresource Technology, 102(22): 10377–10385

McCarty P L, Bae J, Kim J (2011). Domestic wastewater treatment as a net energy producer—Can this be achieved? Environmental Science & Technology, 45(17): 7100–7106

Miyazaki M, Sakai S, Ritalahti K M, Saito Y, Yamanaka Y, Saito Y, Tame A, Uematsu K, Lffler F E, Takai K, Imachi H (2014). *Sphaerochaeta multiformis* sp. nov., an anaerobic, psychrophilic bacterium isolated from subseafloor sediment, and emended description of the genus Sphaerochaeta. International Journal of Systematic and Evolutionary Microbiology, 64(Pt 12): 4147–4154

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–e11

Rotaru A E, Shrestha P M, Liu F, Markovaite B, Chen S, Nevin K P, Lovley D R (2014b). Direct interspecies electron transfer between Geobacter metallireducens and Methanosarcina barkeri. Applied and Environmental Microbiology, 80(15): 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 (2014a). 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(1): 408–415

Sangeetha T, Guo Z, Liu W, Gao L, Wang L, Cui M, Chen C, Wang A (2017). Energy recovery evaluation in an up flow microbial electrolysis coupled anaerobic digestion (ME-AD) reactor: Role of electrode positions and hydraulic retention times. Applied Energy, 206: 1214–1224

Sass H, Ramamoorthy S, Yarwood C, Langner H, Schumann P, Kroppenstedt R M, Spring S, Rosenzweig R F (2009). *Desulfovibrio idahonensis* sp. nov., sulfate-reducing bacteria isolated from a metal (loid)-contaminated freshwater sediment. International Journal of Systematic and Evolutionary Microbiology, 59(Pt 9): 2208–2214

Shen L, Zhao Q, Wu X, Li X, Li Q, Wang Y (2016). Interspecies electron transfer in syntrophic methanogenic consortia: From cultures to bioreactors. Renewable & Sustainable Energy Reviews, 54: 1358–1367

Shimada T, Zilles J, Raskin L, Morgenroth E (2007). Carbohydrate storage in anaerobic sequencing batch reactors. Water Research, 41 (20): 4721–4729

Smith A L, Stadler L B, Cao L, Love N G, Raskin L, Skerlos S J (2014). Navigating wastewater energy recovery strategies: A life cycle comparison of anaerobic membrane bioreactor and conventional treatment systems with anaerobic digestion. Environmental Science & Technology, 48(10): 5972–5981

Speece R E (1996). Anaerobic Biotechnology for Industrial Wastewaters. Nashville: Archae Press

Storck T, Virdis B, Batstone D J (2016). Modelling extracellular limitations for mediated versus direct interspecies electron transfer. ISME Journal, 10(3): 621–631

Strmpl C, Tindall B J, Jarvis G N, Lnsdorf H, Moore E R B, Hippe H (1999). A re-evaluation of the taxonomy of the genus *Anaerovibrio*, with the reclassification of *Anaerovibrio glycerini* as *Anaerosinus glycerini* gen. nov., comb. nov., and *Anaerovibrio burkinabensis* as *Anaeroarcus burkinensis* [corrig.] gen. nov., comb. nov. International Journal of Systematic Bacteriology, 49(Pt 4): 1861–1872

Summers Z M, Fogarty H E, Leang C, Franks A E, Malvankar N S, Lovley D R (2010). Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. Science, 330 (6009): 1413–1415

Summers Z M, Fogarty H E, Leang C, Franks A E, Malvankar N S, Lovley D R (2010). Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. Science, 330 (6009): 1413–1415

Verstraete W, Van de Caveye P, Diamantis V (2009). Maximum use of resources present in domestic “used water”. Bioresource Technology, 100(23): 5537–5545

Wang H, Yang Y, Keller A A, Li X, Feng S, Dong Y, Li F (2016). Comparative analysis of energy intensity and carbon emissions in wastewater treatment in USA, Germany, China and South Africa. Applied Energy, 184: 873–881

Wang L, Liu W, He Z, Guo Z, Zhou A, Wang A (2017). Cathodic hydrogen recovery and methane conversion using Pt coating 3D nickel foam instead of Pt-carbon cloth in microbial electrolysis cells. International Journal of Hydrogen Energy, 42(31): 19604–19610

YangS Y, Zheng Y, Huang Z, WangX M, Yang H (2016). *Lactococcus nasutitermitis* sp. nov. isolated from a termite gut. International Journal of Systematic and Evolutionary Microbiology, 66(1): 518–522

Zhao Z, Zhang Y, Li Y, Dang Y, Zhu T, Quan X (2017). Potentially shifting from interspecies hydrogen transfer to direct interspecies electron transfer for syntrophic metabolism to resist acidic impact with conductive carbon cloth. Chemical Engineering Journal, 313: 10–18

Zhao Z, Zhang Y, Woodard T L, Nevin K P, Lovley D R (2015).

Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials. Bioresource Technology, 191: 140–145

Zhilina T N, Appel R, Probian C, Brossa E L, Harder J, Widdel F, Zavarzin G A (2004). *Alkaliflexus imshenetskii* gen. nov. sp. nov., a new alkaliphilic gliding carbohydrate-fermenting bacterium with propionate formation from a soda lake. Archives of Microbiology, 182(2-3): 244–253