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Case Study

Redox-active biochar facilitates potential electron tranfer between syntrophic partners to enhance anaerobic digestion under high organic loading rate

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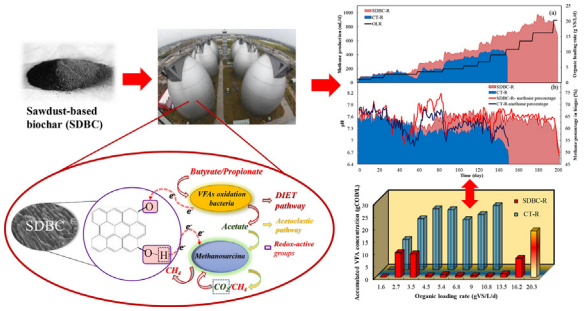
GRAPHICAL ABSTRACT



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ABSTRACT

Sawdust-based biochar prepared (SDBC) at three pyrolytic temperatures were compared as additives to mesophilic anaerobic digestion (AD). SDBC prepared at 500 °C performed better in enhancing CH_4 production than other SDBCs. Analyzing the crucial electro-chemical characteristics of the SDBCs revealed that the excellent electron transfer capacity of SDBC was significant to stimulate methanogenesis promotion. A long-term semi-continuous operation further confirmed that adding SDBC to AD system increased the maximum organic loading rate (OLR) from 6.8 g VS/L/d to 16.2 g VS/L/d, which attributed to the extremely low volatile fatty acids (VFA) accumulation. Microbial community succession analysis found that SDBC addition altered both bacterial and archaea structure greatly. More importantly, the syntrophic and electro-active partners of *Petrimonas* and *Methanosarcina* synergistically enriched under high OLR condition were responsible for the high-efficient VFA degradation, which suggested that SDBC likely acted as redox-active mediator to facilitate direct interspecies electron transfer between the syntrophic partners for high-efficient syntrophic methanogenesis process.

1. Introduction

Currently, anaerobic digestion (AD) technology is considered as an effective strategy for bio-waste treatment, due to its sustainability, low energy consumption, and renewable energy recovery (Mao et al., 2015). However, a challenging issue limiting energy recovery efficiency

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from degradable bio-waste is the excessive accumulation of volatile fatty acids (VFA, e.g., butyrate, propionate and valerate) under high organic loading rate (OLR) condition, which caused by the unfavorable reaction thermodynamics with hydrogen/formate as electron transfer mediators (Li et al., 2017a).

Recent studies found that transforming traditional interspecies electron transfer (IET) with hydrogen or formate as electron transfer mediators into direct interspecies electron transfer (DIET) could be a feasible strategy to promote syntrophic oxidation of VFA (Lovley, 2017). Generally, the feasible mediator for DIET in methanogenic environments included cellular structures of electro-active microorganisms (Rotaru et al., 2013) and redox-active substances (Cervantes et al., 2010). Besides that, introducing some electrically conductive additives (e.g., granular activated carbon, magnetite, biochar and nano-carbon materials) into AD system was beneficial for DIET enhancement as well (Viggi et al., 2014; Zhao et al., 2016; Chen et al., 2014a). Among these materials, biochar has received much attention due to its eco-compatibility and low cost (Lehmann and Joseph, 2015; Azzi et al., 2019).

The effects of biochar addition on methanogenesis was firstly investigated in co-cultures. It showed that biochar addition promoted the possible DIET of *Geobacter metallireducens* with *Geobacter sulfurreducens* or *Methanosarcina* (Chen et al., 2014b). In an UASB digester with VFA as the substrate, biochar stimulated DIET after an ethanol pre-acclimation process (Zhao et al., 2016). For the anaerobic digestion of kitchen waste and cattle manure, adding vermicompost based biochar into the system largely alleviated acidification, which was attributed to the buffering capacity of biochar (Wang et al., 2017). Our previous studies found that the addition of biochar efficiently shortened the lag time and promoted the CH_4 production rate. Furthermore, VFA degradation achieved under high H_2 partial pressure condition suggesting of DIET stimulation in the presence of biochar (Wang et al., 2018a; Qian et al., 2017).

Despite studies addressing the positive effect of biochar addition on methanogenesis, the potential mechanisms of biochar addition for methanogenesis promotion and the dominant role of biochar in an AD system with long-term operation are still in debates as follows. First, most studies attributed DIET enhancement to the highly electrical conductivity (EC) of additives (Gilberto et al., 2018). Nevertheless, the redox-active organic functional groups, such as quinone and phenazines, enriched at the surface of biochar are also likely to make biochar act as electron transfer mediators to promote DIET during syntrophic metabolism (Beckmann et al., 2016; Zhang et al., 2018). As our previous study reported, biochar addition could promote the maximum OLR during long-term thermophilic AD system (Wang et al., 2019), which was largely attributed to the VFA accumulation mitigation. Even though, due to the concerns of operation costs, most current engineering-scale AD systems are based on mesophilic condition, whereas the effects of biochar addition on a long-term continuous AD system under mesophilic system, and the associated microbial community succession characteristics are still unclear.

To fill these knowledge gaps, in this study, the performance of sawdust-based biochar (SDBC) prepared at different pyrolytic temperature for methanogenic promotion of food waste (FW) and sewage sludge (SS) co-digestion was compared to optimize the pyrolytic temperature for biochar preparation and elucidate the potential mechanisms. Thereafter, the optimal SDBC was added into the semi-continuous mesophilic AD system over half year operation with step-wise increase in OLR. CH_4 production efficiency, VFA accumulation conditions, were compared between the SDBC-added reactor (SDBC-R) and the Control reactor (CT-R), and key syntrophic partners for high-efficient methanogenesis under high OLR was also confirmed.

2. Materials and methods

2.1. SDBC preparation and characteristics analysis

The sawdust waste as feedstock for SDBC preparation was obtained with a low cost from a timber mill factory. The pyrolytic temperature for SDBC production were 300 °C, 500 °C and 700 °C (named as SDBC300, SDBC500 and SDBC700), respectively. The particle size of SDBC prepared is 0.25–1 mm. The methods of SDBC preparation were according to our previous study (Wang et al., 2018a). The American Society of Testing Materials (ASTM) methods were used to determine the yields and proximate analysis of SDBC. SDBC was suspended in deionized water with a dosage of 50 g/L, and shaken under room temperature for 2 h. After that, the pH of suspension represented the pH of SDBC. Brunauer-Emmett-Teller (BET) specific surface area of SDBCs were conducted by a surface area analyzer (V-Sorb X800, Gold APP., China). An isotope ratio mass spectrometer (IsoPrime100, Elementar, Germany) were used to confirm the elemental composition of SDBCs. X-ray diffraction (XRD) (Rigaku, Japan) was used for the crystallographic structure analysis. Fourier transform infrared spectroscopy (FT-IR, ThermoFisher, USA) were used to evaluate the organic functional groups on the surface of SDBCs. Electrical conductivity (EC) of SDBC powders were measured with a resistivity tester (ST2722, Jingge, China). A field emission Scanning Electron Microscope (FEI, quanta F50, USA) was used to observe the morphological structures of pristine SDBC and the microorganisms attached on SDBC during the reaction. Electron transfer capacity of biochar were quantified according to the mediated electrochemical reduction (MER)/oxidation (MEO) methods (Zhang et al., 2018). The specific method is described detailed in SI.

2.2. Substrates and seed sludge

The substrates used for AD were mixture of SS and FW. SS was collected from a sludge dewatering unit of local municipal wastewater treatment plant. The synthetic FW was prepared according to the real FW composition of a student canteen in a local university (Wang et al., 2018a). The ratio of SS and FW was 4:1 (based on wet weight) (Dai et al., 2013). The inoculum for AD were collected from an anaerobic digestion treatment unit of a brewery factory in Xi'an. The characteristics of substrate and inoculum were shown in SI.

2.3. Batch experiments of SDBC addition for methanogenesis

Batch experiments were conducted in the 120 mL serum bottles. SDBC300, SDBC500 and SDBC700 were added into the bottle with a 15 g/L dosage, respectively (Wang et al., 2018a). Another group without SDBC addition was operated as control. For each bottle, 5 mL substrate and 85 mL seed sludge were added to keep reaction volume at 90 mL. After that, each bottle was purged with high-pure nitrogen gas for 3 min and then rapidly sealed it with rubber stoppers and aluminum cap to guarantee the anaerobic reaction environment. Lastly, the reactor was put into a 35 °C water-bath shaker with 120 rpm for mesophilic reaction. Biogas production was measured at least once every two days. The experiment was conducted duplicate.

The modified Gompertz equation was used to fitting the average experimental data of each group as shown below:

$$P = P_0 \cdot \exp\left\{-\exp\left[\frac{R_{max} \cdot e}{P_0} \cdot (t_0 - t) + 1\right]\right\}$$

where P was CH_4 production (mL), P_0 was CH_4 production potential (mL), R_{max} was the maximum CH_4 production rate (R_{max}) (mL/d), t_0 is the lag time (days), and $e = 2.718281828$. Origin Pro2018 (OriginLab Corporation, USA) was used to fit CH_4 production process results.

2.4. Long-term semi-continuous experiments

Although batch experiments demonstrated the promotion of methanogenesis after SDBC added, the performance of SDBC addition for a complex long-term operating AD system should be evaluated comprehensively in order to quantify the bonus of SDBC addition for OLR

increase. For this purpose, two AD reactors were designed and operated under continuous (daily-feeding) operation with a reaction volume of 150 mL, which were the SDBC500 (15 g/L) added reactor (SDBC-R) and control reactor without SDBC addition (CT-R). For SDBC-R, particular mass pristine SDBC500 were replenished twice a week to keep consistent SDBC concentration in SDBC-R (Wang et al., 2019). For each reactor, biogas volume was measured by glassy syringe 1–3 times per day (based on daily biogas production volume). After that, 400 μL biogas was collected to measure its composition. At same time point of each day, the reactor was opened to achieve digestate discharging and fresh substrate feeding. The operation process is as same as mention in Section 2.3. The OLR variation of AD system was controlled based on hydraulic retention time variation.

2.5. Analytical methods

The biogas components were daily measured with a gas chromatograph-thermal conductivity detector (GC-TCD) (GC7900, Tianmei, China). The pH of AD reactors were monitored by a potable pH meter (Horiba, Japan). VFA composition were measured with a GC-flame ionization detector (FID) (PANNO, China), which equipped by DB-FFAP column (Agilent, USA) (Wang et al., 2018a).

2.6. Microbial community analysis

The microbial community of sludge sample in reactors were analyzed with high through-put sequencing. Firstly, sludge samples were centrifuged and then rinsed with phosphate buffering saline. After that, an extraction kit (PowerSoil® DNA Isolation Kit, USA) was applied for genomic DNA extraction. The extracted DNA samples were stored in −20 °C environment before further analysis.

For bacterial community sequencing analysis, the V4 regions of 16S rRNA gene was amplified with the primers 515F (5′-GTGCCAGCMGC CGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) via PCR. For archaeal community sequencing analysis, V3-V4 region of 16S rRNA gene were amplified with two cycles. The primers of 1st cycle were 340F (5′-CCCTAYGGGGYGCASCAG-3′) and 1000R (5′-GGCCATGCACYWCYTCTC-3′). The primers of 2nd cycle were 349F (5′-GYGCASCAGKCGMGAAW-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Illumina platform (Illumina Miseq PE250, Sangon Biotech, Shanghai, China) was employed for sequencing analysis (Wang et al., 2018a). According to the results of microbial analysis, principal coordinates analysis was conducted to evaluate the community differentiation and similarity of bacteria and archaea under different OLR condition.

3. Results and discussion

3.1. Optimization of pyrolytic temperature of SDBC preparation for methanogenesis promotion

Biogas production of SDBC300, SDBC500, SDBC700 and Control groups showed that in three SDBC-added groups, CH_4 production was triggered rapidly, whereas a long lag phase occurred in the Control group (Fig. 1). Specifically, SDBC addition shortened the lag time of CH_4 production from 14.0 days to 10.5–6.5 days and increased the R_{max} from 7.6 mL/d to 12.6–13.7 mL/d (Table 1). Among the three biochar-added groups, the fact that SDBC prepared at 500 °C performed much better in lag time shortening and R_{max} increasing than other SDBCs suggested that SDBC produced under a medium pyrolytic temperature could be an optimal choice for CH_4 production enhancement (Table 1). Therefore, the optimal SDBC produced at 500 °C were used further for the semi-continuous long-term operation.

The differentiation of methanogenesis promotion efficiency of SDBC-added groups was likely associated with the physio-chemical characteristics of SDBC, especially the electro-chemical properties. XRD

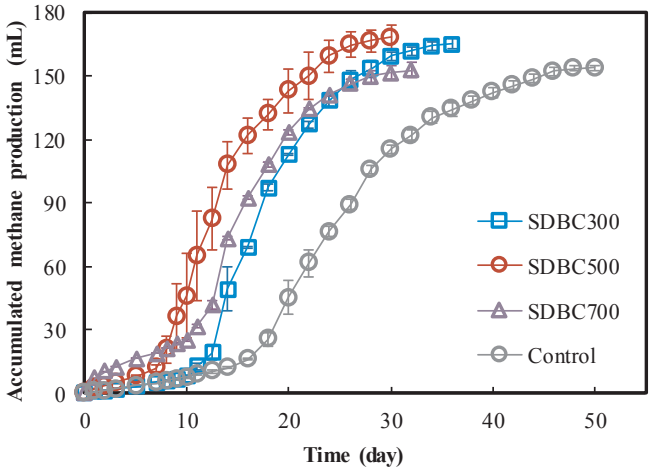

Fig. 1. Effect of pyrolytic temperature of SDBC on accumulated CH_4 production.

Table 1
The fitting results of CH_4 production by modified Gompertz equation.

Group	t_0 (day)	R_{max} (mL/day)	P_0 (mL)	R^2
SDBC300	10.5	12.6	167.2	0.998
SDBC500	6.5	13.7	168.9	0.997
SDBC700	7.2	10.2	163.0	0.990
Control	14.0	7.6	157.7	0.997

analysis demonstrated that the existence of broad peak at 2θ of 18°–26° of the three SDBC, which suggested the formation of turbostratic crystallites, which developed from the crystal structure of lignocellulose of sawdust (SI). Another broader peak appeared at 2θ of 40°–47° in SDBC700 could be attributed to the lateral growth of high ordered graphene planes, which likely contributed to the high EC of SDBC (SI) (Keiluweit et al., 2010). Accordingly, the EC of SDBC increased dramatically as the pyrolytic temperature rising, which accompanying with the increase of C/H ratio (SI). However, the much lower EC of biochar than typically conductive material and better performance of SDBC500 than SDBC700 for methanogenesis promotion (SI) delivered novel insights that EC of SDBC was not a determining factor to accelerate syntrophic methanogenesis process between bacteria and archaea (Chen et al., 2014b).

FT-IR analysis found that SDBC500 was enriched with specific redox-active functional groups. For example, the quinone moiety (at 1557–1567 cm^{-1}) and phenazine (at 1162 cm^{-1}) which likely promoting DIET, whereas few aromatic functional groups were detected in SDBC700 (Klüpfel et al., 2014; Stammer and Taurins, 1963). Consistently, the SDBC500 performed redox-based higher electron transfer capacity than the SDBC300 and SDBC700 (6.67umol e^-/g vs. 0.52–1.74 umol e^-/g), which suggested that for biochar, the redox-active property is more significant than the EC for methanogenesis promotion (SI) (Zhang et al., 2018). Moreover, the alkaline pH of SDBC500 and SDBC700 were beneficial for buffering the pH decrease caused by pre-acidification before CH_4 production (Wang et al., 2018a) (SI). The higher specific surface area of SDBC500 and SDBC700 could support the attached growth of some functional microorganisms to promote methanogenesis (Lü et al., 2016) (SI). These evidences also proved the better performance of SDBC500 and SDBC700 for methanogenic promotion compared to that of SDBC300.

3.2. Effect of SDBC addition on long-term AD performance improvement

3.2.1. Steady CH_4 production under high OLR

The long-term operation started with OLR of 1.1 g VS/L/d, and then

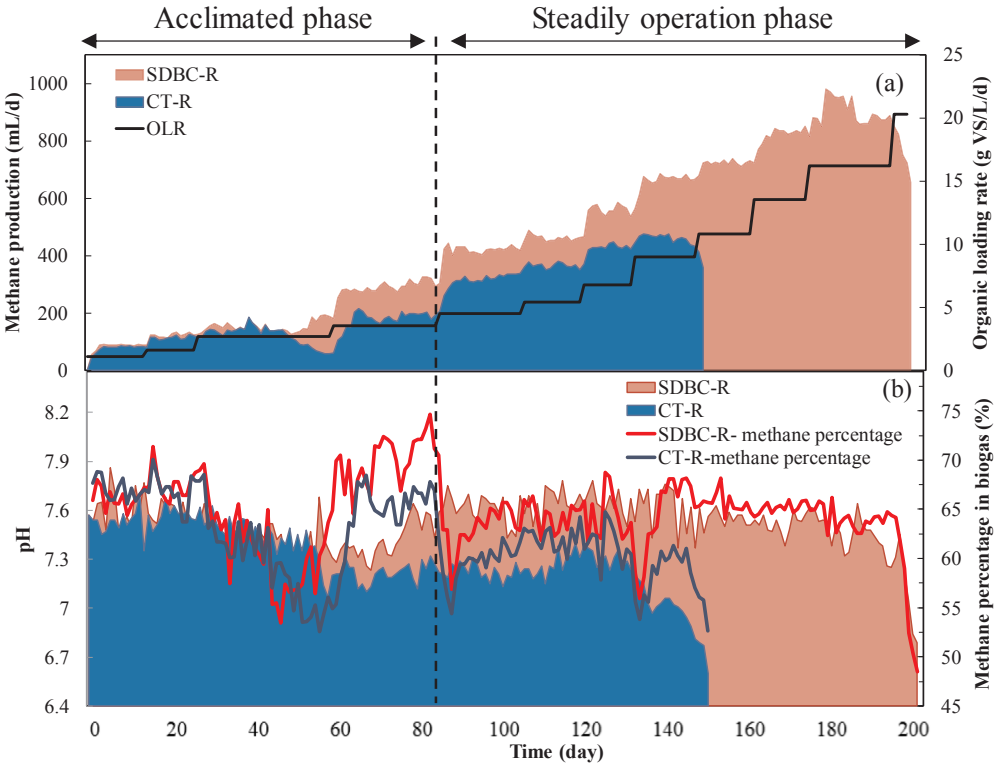

Fig. 2. Variations of daily CH_4 production volume (a), pH and methane percentage of SDBC-R and CT-R (b) as OLR variation.

increase stepwise with hydraulic retention time decrease. As Fig. 2(a) illustrated, the daily CH_4 production differentiation between SDBC-R and CT-R during the OLR increasing from 1.1 g VS/L/d to 3.5 g VS/L/d was negligible. Interestingly, the pH of system and CH_4 content in biogas of both SDBC-R and CT-R showed a slight fluctuation, which suggested that during this phase, the microorganisms in SDBC-R and CT-R went through an acclimated duration. Considering that the source of seed sludge came from an AD unit with brewery wastewater as substrate, great change of substrate characteristics likely caused in the microbial succession in initial stage of operation under low OLR. As OLR increased to 4.5 g VS/L/d, the pH and CH_4 content in biogas of SDBC-R and CT-R kept stable. This indicated that acclimated microbial community has been formed to adapt the substrate of FW/SS, and achieve stable metabolism with this substrate. Therefore, the whole long-term operation of AD system was divided into two phases according to OLR: the acclimated phase with OLR between 1.1 g VS/L/d to 3.5 g VS/L/d and the steadily operation phase with OLR higher than 3.5 g VS/L/d.

During the steadily operation phase (Fig. 2(a)), it is clear that the daily CH_4 production in SDBC-R is higher than that of CT-R. For example, the average CH_4 production of SDBC-R was 292.0 mL/d, which was 60.4% higher than that of CT-R. Further OLR increase enlarged the gap of CH_4 production between SDBC-R and CT-R. Meanwhile, the pH and CH_4 content in biogas of SDBC-R was higher than that of CT-R (Fig. 2(b)). When promoting OLR to 9.0 g VS/L/d, the daily CH_4 production of CT-R performed a dramatically decrease trend due to the pH decrease, whereas the methanogenic activity of SDBC-R was not inhibited before OLR raised as high as 16.2 g VS/L/d. These results indicated the great improvement of CH_4 production with SDBC assistance during a long-term operation of continuous CSTR AD system.

3.2.2. Mitigation of VFA accumulation under high OLR

The characteristics of accumulated VFA of SDBC-R and CT-R illustrated in Fig. 3 could also be analyzed separately based on the acclimated phase and steadily operation phase. During acclimated phase, the VFA accumulation occurred in both SDBC-R and CT-R as OLR increased to 2.7 g VS/L/d. However, the similar accumulation trend demonstrated that the positive impact of SDBC addition for VFA syntrophic degradation promotion is insignificant. As stable microbial community formed, the total VFA accumulated concentration in SDBC-R showed a decrease trend at OLR of 3.5 g VS/L/d until almost no VFA accumulating in system. After that, the total VFA concentration in SDBC-R maintained at a low level (< 2gCOD/L) during the steadily operation phase, until the OLR reach as high as 16.2 g VS/L/d. In contrast, the accumulated VFA concentration of CT-R kept higher than 15 gCOD/L during the steadily operation phase. Although the stable CH_4 production and pH indicated that this concentration range of VFA accumulating is acceptable for methanogenesis process in CT-R because of the adequate alkalinity produced during methanogenesis, the acidification occurred at OLR as low as 9.0 g VS/L/d compared with SDBC-R highlighted the significance of SDBC addition for the promotion of VFA degradation.

For specific VFA type, propionate was the mainly accumulated VFA, which accounted for higher than 70% of the total VFA content (Fig. 3). As a troublesome issue of AD system operation, excessive accumulation of propionate had been reported extensively (Li et al., 2017b). In a recent work, the stimulation of dry AD of municipal solid waste was achieved with the addition of conductive GAC and carbon cloth, while no significant propionate accumulation mitigation was achieved (Dang et al., 2017). In this study, an obvious decrease trend of propionate concentration performed in SDBC-R rather than that in CT-R. For butyrate and valerate, unlike the slightly accumulation that occurred in CT-R, these two VFA were degraded completely during the stable operation of SDBC-R. The acetate concentration of all the reactors maintained at a low level when the reactors operated steadily, which suggested that acetoclastic process rather than syntrophic oxidation was the dominant degradation pathway for acetate. However, a rapid accumulation of acetate, which accompanied by a decrease of pH and drop of daily CH_4 production in both SDBC-R and CT-R, indicated that heavy inhibition of the acetoclastic pathway could be fatal for

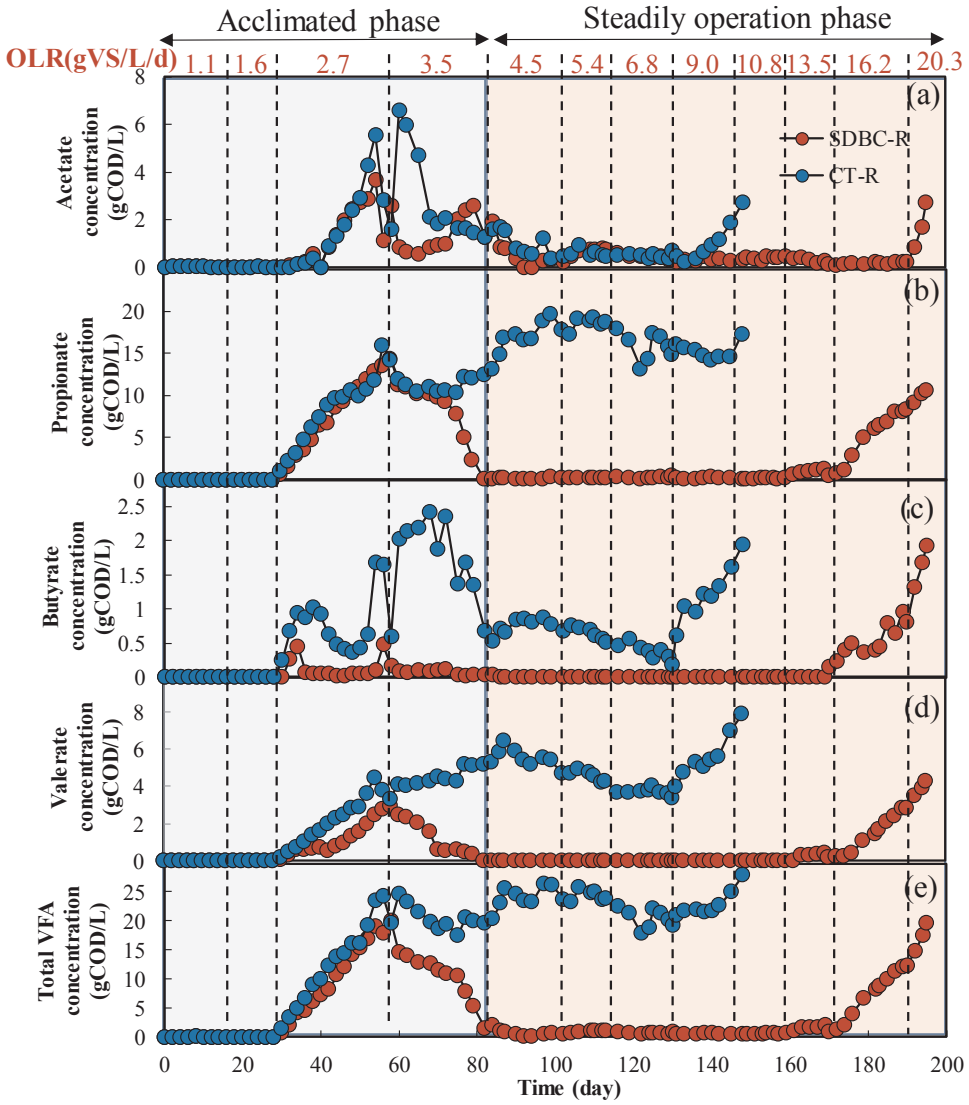

Fig. 3. Concentration variations of acetate (a), propionate (b), butyrate (c), valerate (d) and total volatile fatty acids (e) of the reactors.

methanogenesis, thereby causing the acidification of AD system.

Accordingly, the potential mechanisms of SDBC addition for CH_4 production promotion were analyzed as follows. First, the reversible electron transfer capacity of electro-active oxygen functional groups (quinone, phenazine) on SDBC surface promoted potential DIET for VFA degradation (Prado et al., 2019). Moreover, the developed sheet-like structures and macro-pores of SDBC promoted sphere-like micro-organisms attached growth, demonstrated the function of selective enrichment of microbes of SDBC (Dang et al., 2016) (SI). Noticeably, although the alkali biochar was thought to strengthen the pH buffering capacity of biochar for the stable operation of AD system (Wang et al., 2017), a similar range of accumulated VFA (around 20gCOD/L) causing acidification of the two reactors indicated that the pH buffering capacity contribution of SDBC to AD system under high OLR condition was actually negligible.

3.3. Microbial community characteristics

3.3.1. General description

Both bacterial and archaea communities were analyzed based on high through-put sequencing results. For the bacterial community (SI), *Firmicutes*, *Bacteroidetes*, *Chloroflexi* and *Synergistetes* were the main kinds of bacteria (under phylum level) shared by SDBC-R and CT-R. Generally, these bacteria were considered as commonly fermentative bacteria which existed in AD system (Wang et al., 2018b). However, SDBC addition increased the relative abundance of *Bacteroidetes* and *Chloroflexi* from 18.9% and 4.6% to 34.7% and 11.0%, and decreased *Firmicutes* from 67.4% to 45.4%, respectively (SI), which demonstrated the benefits of SDBC addition for the enrichment of *Bacteroidetes* and *Chloroflexi*.

Archaeal community analysis found that *Methanosarcina*, *Methanobacterium*, *Methanoculleus*, *Methanomassiliicoccus* and *Methanothrix* are main archaeal genus during this process. Typically, *Methanothrix* metabolize acetate with acetoclastic pathway for CH_4 production strictly. *Methanosarcina* is a mixotrophic archaea which could produce CH_4 via both syntrophic pathway and acetoclastic pathway. The other four archaea are typical syntrophic methanogenic archaea (SI).

3.3.2. Comparison of syntrophic partners between SDBC-R and CT-R

The differentiation ($p < 0.05$) of dominant genus between SDBC-R and CT-R under same OLR condition was compared to clarify the effect of SDBC addition on microbial community (Fig. 4). Considering the major aim of this study, differentiation of key microbial community related to syntrophic metabolism and methanogenesis process were analyzed here.

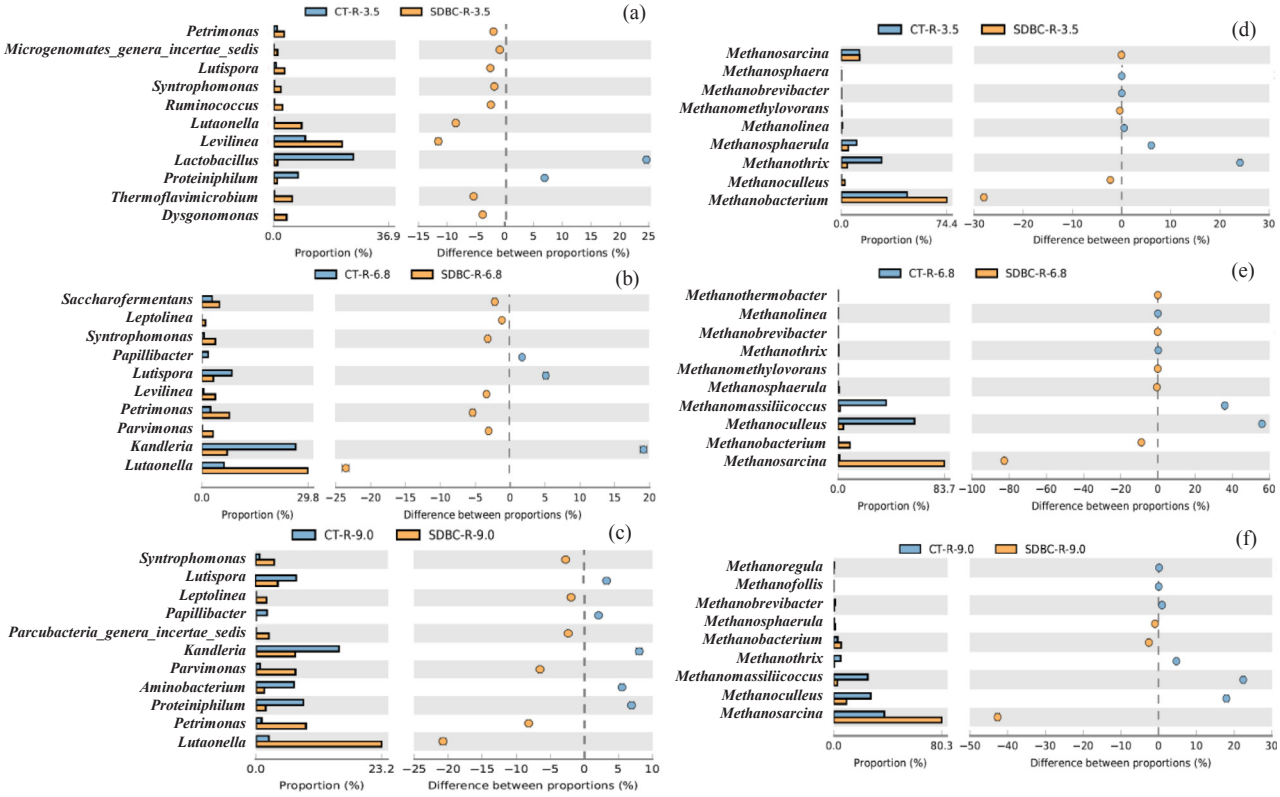

Fig. 4. Comparison of bacteria (a–c) and archaea (d–f) relative abundance differences between SDBC-R and CT-R under same OLR.

For microbial community, *Petrimonas* and *Syntrophomonas* were two main bacteria with the ability of syntrophic metabolism found in CT-R and SDBC-R. Noticeably, during both acclimated phase (OLR of 3.5 g VS/L/d) and steadily operation phase (OLR of 6.8–9.0 g VS/L/d), the relative abundance of *Petrimonas* and *Syntrophomonas* kept at 3.3–9.3% and 2.3–3.8% in SDBC-R, which is 3.0–8.5 and 4.3–11.2 times higher than that in CT-R, respectively. This demonstrated the positive impacts of SDBC addition on these two genera enrichment. As two typical syntrophic bacteria, besides metabolizing with H_2 as electron transfer mediator, some evidences suggested the capacity of DIET of both *Petrimonas* and *Syntrophomonas*. (Grabowski et al., 2005) found that *Petrimonas* could not only metabolize the organic substrates for acetate production, but also transfer electron to the electron acceptor of sulfur for reduction via DIET. In a recent study, it was reported the enrichment of *Petrimonas* in an engineering-scale AD system with GAC assistance, and the authors declared that *Petrimonas* enrichment is responsible for the establishing of DIET (Zhao and Zhang, 2019). What's more, *Syntrophomonas* metabolizes with VFA as substrate (Mcinerney et al., 1981). The enrichment of *Syntrophomonas* in SDBC-R is beneficial for syntrophic oxidation acceleration of VFA. Moreover, in a methanogenic system, it was reported that ferroferric oxide addition stimulated the enrichment of *Syntrophomonas*, which suggested the possible capacity of DIET in this genus (Zhao et al., 2017a).

For archaeal community, *Methanobacterium* (46.4–76.5%) was the dominant genus in SDBC-R and CT-R during acclimated phase. However, great differentiation between SDBC-R and CT-R in steadily operation phase uncovered the influence of SDBC addition on archaeal community and associated methanogenic pathway. For the CT-R, as the OLR increased to 6.8 g VS/L/d, the relative abundance of *Methanobacterium* and *Methanosarcina* decreased from 46.4% to 0.4% and 12.8% to 1.0%, respectively, and *Methanoculleus* (60.1%) and *Methanomassiliicoccus* (37.7%) became the dominant archaea. As the OLR increased further to 9 g VS/L/d, although the relative abundance of *Methanosarcina* increased, the domain archaea in CT-R still were *Methanomassiliicoccus* and *Methanoculleus* (Fig. 4). It seemed that during the operation of CT-R under a high OLR, the two hydrogentrophic methanogens, *Methanoculleus* and *Methanomassiliicoccus*, were the dominant archaeal genera, whereas the high VFA concentrations suggested these two dominant genera could not achieve efficient CH_4 production via conventional hydrogentrophic pathway. For the SDBC-R, an extensive increase of mixotrophic *Methanosarcina* (from 12.9% to 83.7%) was achieved as the reacting process transferring from acclimated phase to steadily operation phase (OLR between 6.8 and 9.0 g VS/L/d), while the *Methanoculleus* (1.6%-4.1%) and *Methanomassiliicoccus* (0.7%-1.7%) maintained a low abundance. Consequently, it is obvious that SDBC addition had a great influence on the microbial community associated with syntrophic metabolism and methanogenesis process.

3.3.3. Key syntrophic partners for stable methanogenesis under high OLR in SDBC-R

To identify the significance of key genera of SDBC-R for the high-efficient VFA oxidation and methanogenesis process, relationship between relative abundance of syntrophic bacteria or methanogenic archaea and accumulated VFA concentration under different OLR of SDBC-R were analyzed. As Fig. 5(a) illustrated, at acclimated phase, the low relative abundance of *Petrimonas* accompanied with average VFA accumulation at 9.5gCOD/L. When the OLR increased to 5.4–10.8 g VS/L/d, the high relative abundance of *Petrimonas* maintained 7.8–13.4%, which were in agreement with the performance of high-efficient VFA oxidation and methane yield during this phase. Further increase OLR to 16.2 g VS/L/d caused relative abundance of *Petrimonas* decreasing from 13.4% to 2.9% drastically, which likely responsible for the occurrence of VFA accumulation at this condition in SDBC-R. Similarly, although the relative abundance of *Syntrophomonas* was lower than that of *Petrimonas* at the same OLR, its variation trend showed negative correlation with accumulated VFA concentration. This result implied the significance of these two syntrophic genera to the high-efficient VFA

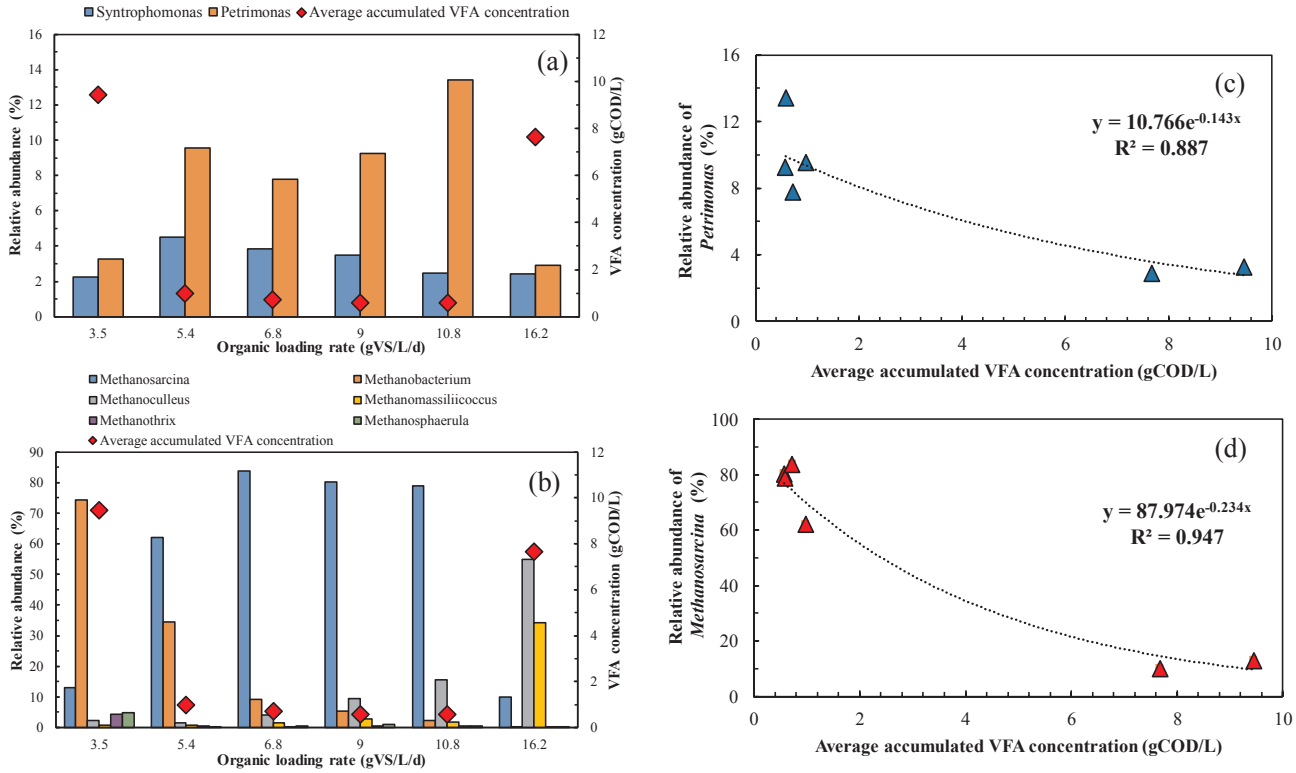

Fig. 5. Relative abundance variation of syntrophic bacteria (a) and methanogenic archaea (b) with OLR increasing in SDBC-R, and the exponential relationship analysis between average accumulated VFA concentration and the relative abundance of syntrophic partners under different OLR (c, d).

degradation.

The trend of different kinds of methanogenic archaea variation based on OLR also unlocked the key methanogens for stable methanogenesis without VFA accumulation (Fig. 5(b)). During OLR at 3.5–6.8g VS/L/d, the average accumulated VFA concentration in system went through a decrease trend from 9.5 to 0.7 g COD/L, thereby the dominant methanogen transferred from *Methanobacterium* to *Methanosarcina*. Thereafter, stable operation with low accumulated VFA accumulation accompanied with *Methanosarcina* enrichment Obviously, *Methanosarcina* was confirmed to be as the key methanogen for high-efficient VFA degradation and methanogenesis under high OLR condition. Compared with the mixotrophic *Methanosarcina*, *Methanobacterium* could only to metabolize VFA with H_2-based syntrophic pathway. However, the low acetate concentration of SDBC-R and CT-R under the whole steadily operation phase suggested that the enrichment of *Methanosarcina* contributed more to the VFA syntrophic oxidation promotion rather than the acetate degradation via acetoclastic pathway. Further increasing OLR to 16.2 g VS/L/d changed dominant methanogen from *Methanosarcina* to *Methanoculleus* and *Methanomassiliicoccus*, and the accumulated VFA concentration started to rise. It seemed that the enriched *Methanosarcina* with SDBC assistance was significant to achieve a highly efficient methanogenesis process and render other methanogens in AD system redundantly, but an overloaded condition likely kicked off it and then cause VFA accumulation. In a previous batch study, it was found that adding biochar into AD system could enrich *Methanosaeta* (*Methanothrix*) firstly and then *Methanosarcina*, which were both important functional archaea for CH_4 production (Lü et al., 2016). However, the result of the present study demonstrated that after a long-term exposure of SDBC, *Methanosarcina* rather than *Methanosaeta* was the key archaea in the biochar amended reactor, which was responsible for the stable operation of high OLRs. Actually, due to the low doubling time, the general strategy for *Methanosarcina* enrichment in AD systems was to decrease the sludge retention time (SRT) to 3–5 days. However, in a CSTR reactor, this could also simultaneously cause the increase of OLR, which lead to severe overloading and VFA accumulation.

The corresponding relationship analysis between relative abundance of *Petrimonas*/ *Methanosarcina* and average accumulated VFA concentration found the strong exponential correlation (R^2 = 0.887 and 0.947) (Fig. 5(c, d)), which further confirmed that the enrichment of these two syntrophic microorganisms is important for high-efficient VFA degradation. Therefore, it was reasonable to postulate that with the redox-active functional groups in surface of SDBC as electron transfer mediator, DIET between *Petrimonas* and *Methanosarcina* was likely stimulated to achieve the VFA oxidation acceleration and rapid methanogenesis, especially under high OLR condition.

3.4. Significance of this study

To address the significance of this study in the aspect of high-efficient renewable energy recovery, the current results was compared with other related reports (continuous-based operation) comprehensively in Table 2. On one hand, compared with the other AD system with same substrate, SDBC addition largely enhanced CH_4 yield and promoted the maximum OLR of AD system. For example, In a high-frequency feeding AD system with FW/SS as substrate, the maximum OLR and CH_4 yield were 11.1 g VS/L/d and 288–350 mLCH_4/gVS, which were 45.9% and 29.5% less than of our study, respectively (Li et al., 2017a). Moreover, the highly conductive and expensive materials used as additive for methanogenesis promotion almost showed a CH_4 yield improvement < 30% (comparing with the corresponding control) (Table 2). However, our study proved that the addition of poorly conductive but redox-active SDBC into AD system showed a comparable CH_4 yield improvement (27.5–48.8%). it believed that the redox-based DIET caused by biochar could outperform other conductive materials, which assisted DIET with the conductive-based model for VFA syntrophic degradation. Besides the benefits of high-efficient renewable energy recovery of AD system, the pyrolysis process for biochar

Table 2
Comparison of reactor performance in this study and other studies.

Substrate	Feeding style	HRT (day)	(Maximum) OLR (g VS /L·day^{-1})	CH_4 yield (mL CH_4/g VS)	Electrically conductive additive	CH_4 yield improvement (%)	Reference
FW + SS (1:2)	Daily-feeding	33–8.3	4	230–260	–	–	(Gou et al. 2014)
FW + SS (4:1–2:3)	Daily-feeding	30–8	15–18.5	215–304	–	–	(Dai et al. 2013)
FW + SS (3:1)	Once per 15 min	33–7.5	11.1	288–350	–	–	(Li et al. 2017a)
FW + SS (1:3–3:1)	4 times per day	20	2.83–6.88[a]	183-268[b]	–	–	(Jang 2016)
FW + SS (4:1)	Daily-feeding	50–5	16.2	373–452	Biochar	27.5–48.8	This study
Dairy effluent	Daily-feeding	25–20	1.02	70.8–124.9[b]	Magnetite	22.8	(Baek et al. 2016)
Ethanol + VFAs	Continuously	0.5	10.28 [a]	290.0–334.6[b]	Biochar	16–25	(Zhao et al. 2016)
Synthetic wastewater	Daily-feeding	2	5.2 [a]	180.3[b]	Carbon cloth	20.4	(Zhao et al. 2017b)
Dairy wastewater	Daily-feeding	4	26.5 [a]	245–341.6[b]	GAC	5.7–31.2	(Zhao et al. 2017c)
Artificial bio-waste	Daily-feeding	10	10.3 [a]	178.2[b]	GAC	6.5	(Dang et al. 2016)
Artificial bio-waste	Daily-feeding	10	8.5 [a]	183.2[b]	Carbon cloth	9.5	(Dang et al. 2016)
Artificial bio-waste	Daily-feeding	10	8.5 [a]	185.2[b]	Carbon felt	10.7	(Dang et al. 2016)
Artificial bio-waste	Daily-feeding	10	6.7 [a]	169.1[b]	Graphite	1.1	(Dang et al. 2016)

Notes: a means the unit is g COD/L/d; b means the unit is mL CH_4/g COD

production was also significant for carbon neutrality, and even carbon negativity achievement for the world. During pyrolysis of bio-waste (mainly refractory substances, e.g., agriculture residues and forest biomass), the extracted syngas and bio-oil under high temperature could be used for combined heat and power (CHP) production. In a recent report, (Azzi et al., 2019) evaluated the impact of biochar production and use in a city-scale on climate change mitigation via life cycle assessment, and found that compared with the current scenarios with forest biomass as energy source, an integrated CHP system with pyrolysis and biochar comprehensive utilization was more beneficial for climate change mitigation, especially in a long-term duration-based evaluation. Therefore, our study highlighted the feasibility of combining two promising bio-waste treatment technologies of pyrolysis and AD together to achieve a win–win strategy in the aspects of renewable energy production promotion and climate change mitigation.

4. Conclusion

The performances of SDBC prepared at different pyrolytic temperatures as additives for methanogenesis promotion were investigated. It was found that SDBC likely acted as potential redox-active mediators to facilitate electron transfer between syntrophic oxidizer and methanogens. A long-term operation experiments confirmed that the optimal SDBC greatly promoted CH_4 yield and increased maximum OLR, which mainly attributed to the acceleration of VFA oxidation. The exponential negative correlation between relative abundances of syntrophic electroactive microorganisms and accumulated VFA concentration addressed the significance of SDBC addition on syntrophic microbial community enrichment, which is responsible for stable operation under high OLR.

Author Contribution

Gaojun Wang and Qian Li contributed to the experiments design and manuscript writing, Gaojun Wang, Yu Li, Yao Xing, and Gaofei Yao contributed to expeirments operation and manuscript editing, Yanzheng Liu contributed to improve the revised manuscript, Rong Chen and Xiaochang C. Wang contributed to the data analysis.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

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