Bioresource Technology 294 (2019) 122235

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Evaluating the effect of biochar on mesophilic anaerobic digestion of waste activated sludge and microbial diversity

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ARTICLE INFO

Keywords:
Anaerobic digestion
Methane
Hydrochar
Pyrochar
Sludge-derived biochar
Waste activated sludge (WAS)

ABSTRACT

This study compared the effects of sewage sludge-derived pyrochar (PC300, PC500, and PC700) and hydrochar (HC180, HC240, and HC300) on mesophilic anaerobic digestion of waste activated sludge (WAS). It was demonstrated that hydrochar can better promote the methane production compared with pyrochar. The highest accumulative methane yield of 132.04 ± 4.41 mL/g VS_{added} was obtained with HC180 addition. In contrast, the PC500 and PC700 showed a slightly negative effect on methane production. Sludge-derived HC not only accelerated the solubilization and hydrolysis of sludge floc, but also improved the production of acetic acid and propionate, further resulting in improved methane production. Simultaneously, the syntrophic microbes facilitating direct interspecies electron transfer (DIET) such as *Syntrophomonas, Peptococcaceae, Methanosaeta* and *Methanobacterium* bred rapidly with the addition of HCs. These results indicated that the hydrochar is more ideal as the accelerant to promote the methane production from mesophilic anaerobic digestion of WAS than the pyrochar.

1. Introduction

Annually, huge amount of waste activated sludge (WAS) are produced from municipal wastewater treatment plant (WWTP) as the solid byproduct in China and how to dispose them has become a great environmental issue (Wu et al., 2018). Anaerobic digestion (AD) is a promising way to deal with WAS because it can simultaneously achieve the organics degradation and energy recovery. There are great environmental and economic benefits through AD of WAS such as sludge volume reduction, sludge stabilization, nutrient (nitrogen and phosphorus) recycling, and biogas (methane or hydrogen) production (Zhao et al., 2017; Li et al., 2019). However, WAS consists of high-strength macromolecule organics, such as polysaccharide and protein, whose decomposition by microorganisms is quite slow (Bougrier et al., 2006). Meanwhile, AD process is often inhibited by the accumulation of short chain fatty acid (SCFA), ammonia or heavy metal, resulting in the instable digestion process and poor biogas yield (Luo et al., 2019a).

To facilitate the AD and obtain more biogas yields, the addition of accelerants to the digestor was widely employed. The common accelerants include metal accelerants and metal-free accelerants. It is reported that the growth of methanogenic bacteria is dependent on Fe, Co, and Ni during enzyme synthesis (Qiang et al., 2012). Abdelsalam et al. (2016) found the addition of nanoparticles including Co, Ni, Fe and Fe_3O_4 with a moderate concentration had a clear biostimulating effect on the methanogenic activity in the AD of slurry and the methane yield increased 1.67 ~ 2.17 times compared with the control. However, many researches confirmed that the presence of metal materials such as TiO_2, ZnO, and CuO inhibited the hydrolysis, acetogenesis, and methanogenesis in AD process due to the releasing of metal ions (Luna-Delrisco et al., 2011; Mu and Chen, 2011). Recently, a low-cost metal-free accelerant, biochar was applied to stimulate AD process. On the one hand, biochar is a porous carbon material with rich surface functional groups, therefore, it could remove free ammonia and ions by adsorption and ion-exchange to eliminate their negative effects on AD (Yang et al., 2018; Pan et al., 2019). On the other hand, recently, an electron transport mode between bacteria and methanogenic archaea, direct interspecies electron transfer (DIET), has been proven to be effective to promote the syntrophic conversion of various reduced organic compounds to methane in AD (Barua and Dhar, 2017). As a common conductive material, the biochar could serve as conductive nanowire like pili and assist the electrical connection of syntrophic metabolism (Chen et al., 2014a). These properties make the biochar a possible

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https://doi.org/10.1016/j.biortech.2019.122235
Received 13 August 2019; Received in revised form 24 September 2019; Accepted 1 October 2019
Available online 02 October 2019

external accelerant to enhance the methanogenesis.

There are mainly two types of biochar, the pyrochar (PC) produced by dry pyrolysis in an oxygen- free atmosphere and the hydrochar (HC) prepared through the hydrothermal carbonization (HTC) in the presence of subcritical water (Zhou et al., 2018; Luo et al., 2019b). The different preparation conditions make the two kinds of biochar have significantly different physicochemical properties (Kambo and Dutta, 2015). For example, the compressed liquid in the HTC process promotes the demineralization of ash composition from biomass, so the hydrochar usually has lower percentage ash content. Using corn stover as raw material for biochar production, compared to the pyrochar the surface area and porosity of hydrochar was poor (Fuertes et al., 2010). Obviously, these difference between the pyrochar and hydrochar will affect their performance as the additive to promote AD process. To date, the most of researches on AD enhanced by biochar focused on the pyrochar. Yuan et al. (2018) investigated the effects of pyrochars derived from rice straw (RB), manure (MB) and wood chips (WB) on the anaerobic process with ethanol as a substrate. Experimental results showed that RB and MB stimulated methanogenesis by facilitating the DIET between methanogens and *Geobacteraceae,* resulting in 10.7 and 12.3-folds improvement for methane production rate, respectively, while the WB had little improvement on methane production. Pan et al. (2019) found that fruitwood char pyrolysed at 550 °C improved the methane yield to 294 mL CH_4/g VS_{added} from 174 mL mL CH_4/g VS_{added} in the AD of chicken manure. They attributed it to the large specific surface area and high total ammonia nitrogen reduction capacity of as-prepared pyrochar. However, to the best of our knowledge, the role of hydrochar in the enhancement of AD is barely investigated. Meanwhile, the comparison between pyrochar and hydrochar to affect the mesophilic anaerobic digestion performance of WAS and microbial diversity was also paid less attention.

In this study, we prepared PCs pyrolysed at 300, 500 and 700 °C, and HCs by HTC at 180, 240 and 300 °C using WAS as the raw material, respectively. The effectiveness of different types of biochars on the enhancement of mesophilic AD of WAS was evaluated. Simultaneously, microbial community was analyzed using a high-throughput sequencing technology to understand the different enhanced mechanism induced by different types of biochar.

2. Materials and methods

2.1. Substrate and inoculum

The WAS used in this study was collected from the secondary sedimentation tank of a municipal wastewater treatment plant (Changsha, China) and stored in the refrigerator at 4 °C before use. Inoculation sludge was taken from the granular anaerobic sludge reactors in our own laboratory, and the granular sludge had been domesticated for several months. WAS and inoculation sludge was mixed thoroughly at a volume ratio of 5:1. The main characteristics of mixed sludge were: pH 7.10 ± 0.10, total suspended solids (TSS) 19,690 ± 150 mg/L, volatile suspended solids (VSS) 10,460 ± 120 mg/L, soluble chemical oxygen demand (SCOD) 529 ± 22 mg/L, soluble carbohydrate 31.33 ± 0.67 mg/L, soluble protein 75.67 ± 3.13 mg/L.

2.2. Sludge-derived pyrochars and hydrochars

For comparison, both PCs and HCs were respectively prepared in this experiment. The dewatered WAS collected from the same municipal wastewater treatment plant (Changsha, China) was used to prepare the two types of biochars. The dewatered sludge was firstly heated at 105 °C for 48 h in oven to completely remove water content, then grounded and sieved into fine powders (< 0.25 mm) for further use.

Dry sludge powder was pyrolysed in a horizontal furnace with a heating rate of 5 °C/min to a desired final temperature of 300, 500 and 700 °C for 2 h. Nitrogen gas (99.99%, v/v) as the protective gas was supplied to the furnace at a flow rate of 650 mL/min during heating and cooling process. Three PC samples were labeled as PC300, PC500, PC700, respectively. HC was prepared through the HTC. Deionized water was added into dry sludge particles at the mass ratio of 1: 9, then the mixture was loaded into a 500 mL stirred pressure reactor equipped an internal pressure and temperature sensors. The reactor was heated to the desired temperature of 180, 240 and 300 °C and maintained for 2 h. Then the hydrothermal products were cooled to room temperature, dried at 105 °C for 12 h and lastly ground into fine powders (< 0.25 mm), named respectively as HC180, HC240, and HC300. All biochars samples were stored into sealing bags before next use.

2.3. Mesophilic anaerobic digestion of WAS

The batch experiments to evaluate the effect of different biochars on mesophilic anaerobic digestion of WAS were conducted in seven 500 mL serum bottles. Firstly, each bottle was added 250 mL WAS and 50 mL inoculation sludge. Then, 3.00 g PCs produced at different pyrolysis temperature (PC300, PC500, PC700) were added into three bottles, respectively. Simultaneously, 3.00 g HCs prepared at various hydrothermal temperature (HC180, HC240, and HC300) also were fed in other three bottles, respectively. The remaining bottle without the addition of biochar was set as the Blank. The initial pH in all bottles was adjusted to 7.00 ± 0.10 using 4 M HCl or 4 M NaOH. In order to maintain the strict anaerobic environment, all the bottles were purged with pure nitrogen for 2 ~ 3 min to remove the oxygen and immediately sealed with a rubber plug. All bottles were placed in a constant temperature water bath shaker with 150 r/min at 37 °C for the sludge digestion. The experiment was terminated when no obvious biogas yielded from the bottles (about 32 days). All experiments were conducted in triplicate simultaneously to control accidental error.

2.4. Analytical methods

2.4.1. Characterization of biochars

An ASAP 2020 PLUS HD88 instrument (Micromeritics, USA) was used to analyze the Brunauer-Emmett-Teller (BET) surface area, pore volume and size of the biochars. Before analyzing, all samples were degassed at the temperature of 150 °C and vacuum of 500 μmHg for 4 h. The surface functional groups on biochars was detected by Fourier transform infrared (FTIR) spectrometer (Nicolet iN10, Thermo, USA). The biochar-deionized-water solutions at 1:20 (w/v) biochar/water ratio were shaken for 1.5 h then to measure the pH, electrical conductivity (EC) and dissolved organic carbon (DOC) of biochars. pH and EC were detected by the pH device (PHS-3C, INESA, China) and conductivity meter (DOS-307, Yueping, China), respectively. The DOC was determined by TOC analyzer (TOC-V CPH, Shimadzu, Japan).

2.4.2. Chemical analyses

The pH, EC, TSS, VSS, soluble COD (SCOD) of digested substrate in anaerobic reactor were determined followed the standard methods (APHA, 2005). Protein was measured using the Lowery-Folin method with bovine serum albumin (BSA) as the standard. Carbohydrate was measured by the anthrone colorimetric method with glucose as the standard at 620 nm. Nessler reagent spectrometry was used to determine the concentration of total ammonia nitrogen (TAN) in mixed liquid at 420 nm. CH_4 and H_2 in the produced biogas were analyzed using a gas chromatograph (GC112A, INESA, China). SCFAs were analyzed with gas chromatograph (GC-2010, Shimadzu, Japan).

2.4.3. Microbial community analysis

The initial mixture (day 0) and seven samples from seven different bottles at the end of operation (day 32) were collected, which were marked Blank0d, Blank32d, HC180, HC240, HC300, PC300, PC500 and PC700 respectively. The microbial DNA of sludge samples were

extracted by using an extraction kit (Bioteke Corporation, Beijing, China). The V3-V4 region of the bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using a primer set of 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGT-WTCTAAT-3′) for sample identification. The V4-V5 region of the archaeal 16S rRNA gene was amplified via PCR with a primer set of 524F (5′-TGYCAGCCGCCGCGGTAA-3′) and 958R (5′-YCCGGCGTTGAVTCC-AATT-3′) for sample identification. The details about PCR reaction for 16S rRNA genes of bacteria and archaea as follows: Initial denaturation 98 °C 2 min; Denaturation 98 °C 15 s; Annealing 55 °C 30 s; Extension 72 °C 30 s; Final extension 72 °C 5 min; Repeat 25–30 cycles from denaturation to extension; 10 °C hold. PCR amplification products were detected by 2% agarose gel electrophoresis, and the target fragments were cut and recovered using the gel recovery kit of AXYGEN company. Fluorescence quantification was performed on the recovered products amplified by PCR. The fluorescence reagent was from Quan-iT PicoGreen dsDNA Assay Kit, and the quantitative instrument was Microplate reader (BioTek, FLx800). Illumina Miseq-PE250 sequencing service was provided by Personal Biotechnology Co., Ltd. Shanghai, China. Finally, the sequences obtained were clustered into OTUs (operational taxonomic units) at a 97% similarity. Further analyses such as alpha and beta diversity analysis and taxonomic composition analysis were used to illuminate the effects of HC and PC on microbial community of anaerobic digestion of WAS.

2.5. Statistical analysis

The experiment was conducted in triplicate simultaneously and results were expressed as mean standard deviation. An analysis of variance was used to evaluate the significance of results, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Characterization of biochars

Table 1 summarized the physicochemical properties of two types of biochars (PCs and HCs). The characteristics of sludge-derived biochars varied considerably depending on the produced temperature and carbonization method. Different from biochars derived from other carbonaceous organic material (Zhou et al., 2018), sludge-derived HCs in this study had larger specific surface area, total pore volume and pore size compared with PCs (Table 1). It should be attributed to the fact that the precursor WAS mainly consists of flocs though the jointing of extracellular polymeric substance (EPS). These flocs contract strongly under dry pyrolysis, which is adverse to form porous biochar. Contrarily, there are plenty of little bubbles during HTC, which effectively improve the production of porous biochar. Therefore, HC180 exhibited the greatest BET surface area and pore volume (58.01 m^2/g and 0.31 cm^3/g, respectively) in all samples. However, when the hydrothermal temperature further increased to 300 °C, the surface area gradually decreased to 31.16 m^2/g. Unlike HCs, the surface area of PCs increased from 21.32 to 39.76 m^2/g in the temperature range of 300 ~ 500 °C, then declined to 31.78 m^2/g at 700 °C due to the tunnel collapsed.

The pH of original sludge particle was 6.36, which was lower than that of both two types of PC and HCs. Most noticeably of all, the pH values of PCs or HCs gradually rose with the increase of produced temperature. In Yuan et al. (2011), the alkalinity and pH of biochar increased with the increase of pyrolysis temperature, which due to $-COO^-$ (−COOH) and $-O^-$ (−OH) became the major alkaline components in the biochar. In this study, the sludge-derived biochars treated by pyrolysis presented higher alkalinity (PCs; pH 6.78 ~ 7.92) than that treated by HTC (HCs; pH 6.40 ~ 7.08). That may attribute to PC that produced at higher temperature than HCs generated more alkalinity from the conversion of surface functional groups (Jiang and Xu, 2013). The maximum EC value was 1174 μs/cm belonged to the original sludge particle, while HC300 has minimal value of 136.4 μs/cm. When the produced temperature from 300 to 500 °C, EC of pyrochar dropped from 641.0 to 274.0 μs/cm rapidly. Further increasing of pyrolysis temperature to 700 °C, EC slowly decreased to 248.5 μs/cm. Compare to PCs, HCs had lower EC values which was between 136.4 and 268.0 μs/cm. For both of HCs and PCs, EC values decreased with the increase of pyrolysis or hydrothermal temperature. DOC is a critical indicator for the growth of microbial activity on the surface of biochar. The HCs (HC180, HC240 and HC300) and PC300 had a relatively high DOC level (2.17 ~ 5.71 mg/g), while DOC of PC500 and PC700 is only 0.39 and 0.15 mg/g. This suggested that pyrolysis process has a greater impact on DOC than hydrothermal process, and HCs remained more dissolved organic matter than PCs.

It is well-known that pyrolysis and hydrothermal process change the surface functional groups of raw material. FTIR spectra of the raw sludge particle and two types of sludge-derived biochars were all detected (E-supplementary data). All biochars showed the peak at 2360 cm^{-1}, which attributed to the CO_2 adsorption. The peaks at wavenumbers of 3617 cm^{-1} (−OH), 2923 cm^{-1} (C−H), 1612 cm^{-1} (C═C) and 1033 cm^{-1} (C−O−C) showed the surface carbon structure of the HCs and PCs. Compared to PCs, hydrothermal carbonization retained most of surface functional groups of raw material. The adsorption peaks at 2923 and 1612 cm^{-1} were weaken with the increase of hydrothermal temperature, thus indicating the content of C−H and C═C bonds slightly decreased. On the contrary, with the increase of pyrolysis temperature, many surface functional groups (i.g. −OH, C−H) would be destroyed. For instance, −OH bond (3617 cm^{-1}) was vanished at 300 °C, and when the pyrolysis temperature climbed to 500 °C, both −OH and C−H bonds were decomposed. As for PC700, the spectrum was fairly flat suggesting that the functional groups mentioned above was decomposed at a high pyrolysis temperature (Liu et al., 2010).

3.2. Performance of sludge-derived biochars on methane production

The methane production during the whole anaerobic digestion of all reactors are shown in Fig. 1. In the first 8 days, the methane yields in that reactors with HC addition were lower than that of Blank, while no significant difference on methane production was observed between group Blank and PCs. After 32 days of anaerobic digestion, the

Table 1
Physicochemical properties of the biochars.

Characteristics	Sludge	PC300	PC500	PC700	HC180	HC240	HC300
BET Surface Area (m^2/g)	12.6355	21.3196	39.7573	31.7746	58.0149	42.1210	31.1635
Pore volume (cm^3/g) [a]	0.055913	0.090878	0.141885	0.125656	0.307522	0.302335	0.179901
Pore size (nm) [b]	14.1190	10.7554	11.5744	11.3255	15.5457	23.7284	18.4326
pH	6.36	6.78	7.50	7.92	6.40	6.82	7.08
EC (μs/cm)	1174	641.0	274.0	248.5	268.0	185.2	136.4
DOC (mg/g)	22.96	4.90	0.39	0.15	5.71	3.32	2.17

[a] BJH Desorption cumulative volume of pores; P/Po = 0.99.
[b] BJH Desorption average pore diameter (4 V/A).

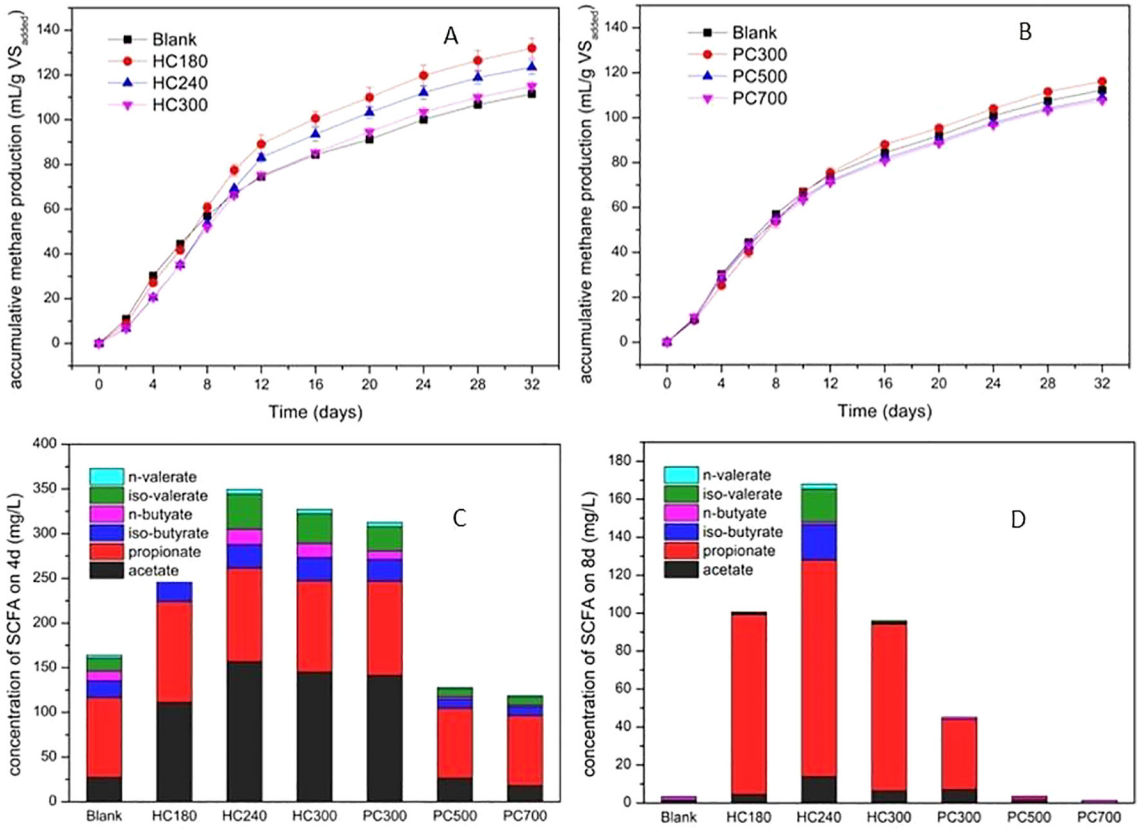

Fig. 1. The profiles of cumulative methane production with hydrochar (A) and pyrochar (B) addition; SCFA concentration and proportion on the 4th day (C) and 8th day (D).

accumulative methane yield was 132.04 ± 4.41, 123.48 ± 1.59, 114.95 ± 3.05 mL/g VS_{added} under the addition of HC180, HC240, HC300 (Fig. 1). Compared with the Blank group (111.52 ± 1.09 mL/g VS_{added}), the methane production exhibited 18.40%, 10.72% and 3.07% increases, respectively. Simultaneously, at the presence of PCs, only group PC300 showed a slight improvement in the accumulative methane production (116.17 ± 0.43 mL/g VS_{added}), PC500 and PC700 both had negative effects on finally methane production. At the 0.05 level, the cumulative methane production in PC300, PC700, HC180 and HC240 ($n = 3$, $p < 0.05$) are significantly different with that in Blank. This indicated that HC addition could accelerate the WAS digestion in the whole anaerobic process and result a higher methane yield, but the methane production was inhibited in the initial stage due to the higher SCFA inhibition with HCs addition in initial phase and inadaptation of methanogens. The detailed discussion of SCFA variation was in Section 3.4. Zhou et al. (2018) investigated the effect of hydrochar and pyrochar derived from saw dust or wheat straw on CH_4 emission from rice paddy. They found that 3% (w/w) pyrochar significant decreased the paddy CH_4 emission, contrarily, 3%-hydrochar stimulated the CH_4 emission, which induced by saw dust and wheat straw-based hydrochar was 4 and 6 times of char-free control treatment respectively. In another research, the biogas yield in digestion of fish processing waste was increased by 64% with a bamboo hydrochar adding ratio of 1:2 (dry mass ratio of fish processing waste to hydrochar) (Choe et al., 2019). As a preliminary study, the significance of this study is great, although the methane yield improvement of HC180 is not very high. This research proved the feasibility of WAS re-utilization and pointed out that sludge-derived hydrochar is an ideal accelerant to be further modified by some works.

3.3. Release and biodegradation of SCOD, protein and carbohydrate

The evolution of SCOD, soluble protein and soluble carbohydrate in mixed liquid could represent the solubilization degree of organic matters in WAS. Fig. 2 shows the variation of soluble organic matters in mixed liquid for various groups through the whole anaerobic process (32 days). In the reactors with HCs addition, during the first 4 days, the level of soluble COD and protein and carbohydrate concentration exhibited an upward trend. It is worth noting that the protein level of HC180 (178.17 ± 20.24 mg/L, highest value in whole process) even reached 1.8 times of Blank (97.13 ± 15.31 mg/L) on day 4. However, the carbonhydrate in HC reactors showed a slight decline before day 4, presumably because of the inhibition to carbonhydrate solubilization with HC addition and the adsorption of HC. In the following days, in HC reactors, the SCOD concentrations gradually stabilized to different levels with little variations until the end of digestion (248.09 ~ 393.13 mg/L). And the soluble protein and carbohydrate concentrations obeyed the sequence of HC180 > HC240 > HC300 > Blank. Compared to the groups with HCs addition, the presence of PC500 or PC700 has a significant inhibition to the release of SCOD, protein and carbohydrate (Fig. 2). Interestingly, the soluble matter concentration in the PC300 presence was significantly higher than that of Blank, especially the soluble protein always keep at a high level (134.42 ~ 171.92 mg/L) from day 4 to the end of operation (Fig. 2). And the group PC300 showed similar trends to group HC180 for the solubilization of substrate. The carbohydrate and protein concentrations in the groups with PCs addition followed the sequence of PC300 > Blank > PC500 > PC700 when the concentration was stable (except for the carbohydrate concentration on day 32).

The results showed that HCs (produced at the temperate range from 180 °C to 300 °C) have better catalytic effects on the solubilization and hydrolysis of WAS than PCs produced at 500 °C and 700 °C, while the

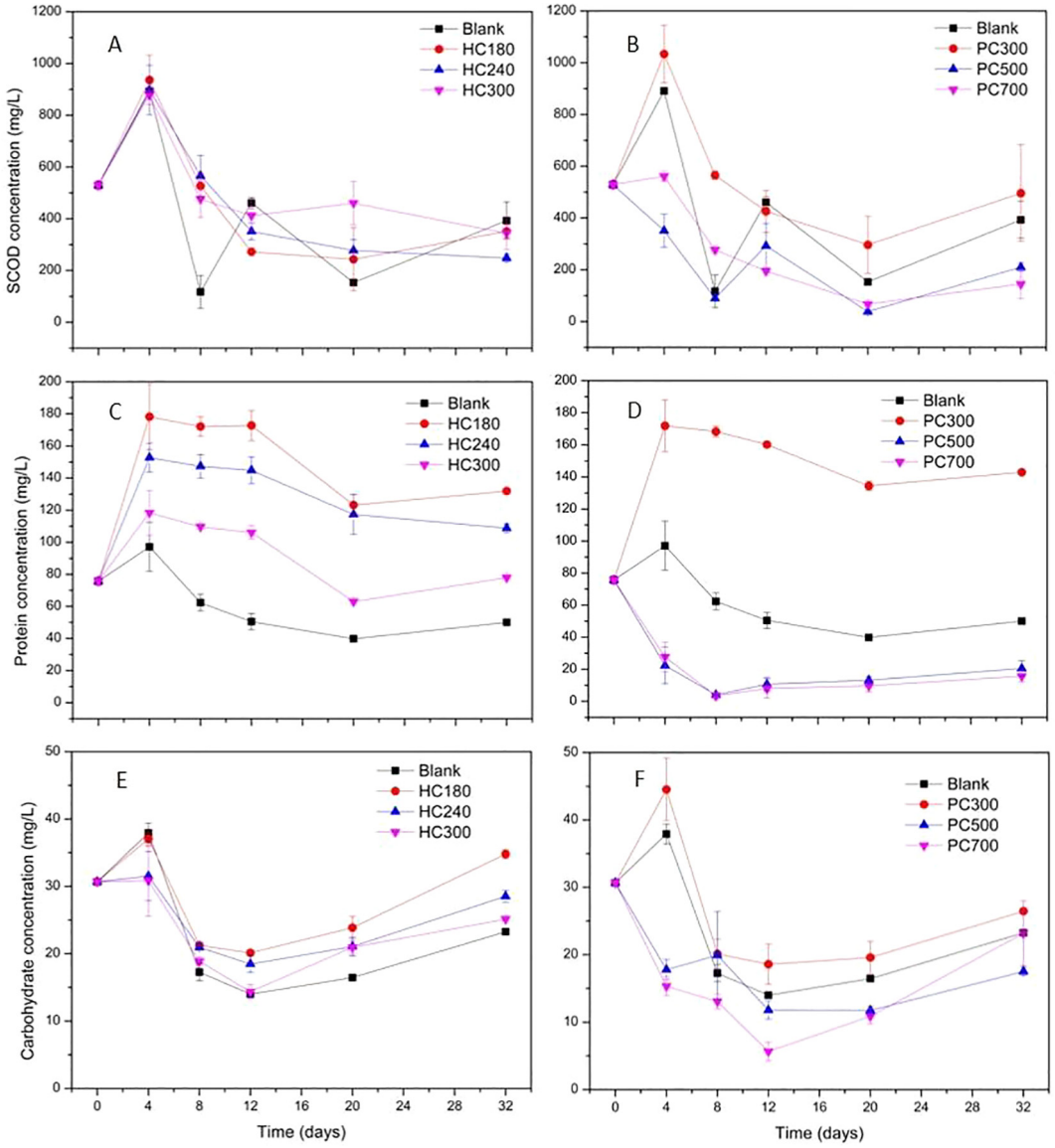

Fig. 2. Effect of hydrochar (A, C, E) and pyrochar (B, D, F) addition on soluble COD, soluble protein and soluble carbohydrate produced from the WAS anaerobic digestion.

PC300 showed a similar performance to HC180. Moreover, the temperature affected the ability of biochars. As the pyrolysis temperature increased, the structure of biochar became porous, and the pore channels were more uniform thus a higher adsorption capacity will be obtained (Chen et al., 2014b). Therefore, the soluble organic matters decrease with the increase of PC produced temperature. However, some surface functional groups were also lost in higher temperature (Peng et al., 2016), which may weaken the ability of ion-exchange (ion-exchange is the predominant adsorption mechanism of HCs) (Liu et al., 2010). This may lead to the similar trend of protein in group PC500 and PC700. Liu et al. (2010) found that pinewood pyrochar produced at 700 °C has higher surface area but lower adsorption ability than hydrochar produced at 300 °C. In our previous studies, sludge-derived HC promoted the solubilization and hydrolysis of organic WAS particles due to the enhancement of activity of hydrolase (Wang et al., 2017). Although HCs has higher surface area and pore volume than PCs (Table 1), the soluble protein and carbohydrate in group HCs still hold on the high level. This indicated that the level of soluble organic matters was mainly determined by the promotion effects of HCs to hydrolysis and acidification.

3.4. Variations of SCFA

SCFA is the most important intermediate substance in anaerobic digestion. The total SCFA consists of six individual acids (i.e. acetate, propionate, iso-butyrate, n-butyate, iso-valerate, and n-velerate). In this study, Fig. 1C and D presents the concentration and proportion of SCFA on 4th and 8th day. It can be seen that HCs (HC180, HC240, HC300) and PC300 affected SCFA concentration and proportion largely. In contrast, PCs (especially PC500 and PC700) had little influence on SCFA composition. The total SCFA levels of PC500 and PC700 were slightly lower than Blank, which indicated that PCs except for PC300 inhibited the SCFA production of WAS digestion system. Notably, the level of total SCFA in group PC300 was similar to that in reactors with HCs addition, however, the pH value of group PC300 was higher than that of Blank and HCs. This could attribute to the alkaline substance from the P300. And the higher alkali and pH was in PC500 and PC700 (Table 1), thereby SCFA production was inhibited.

In the groups with HCs (HC180, HC240, HC300) and PC300 addition, after 4 days the SCFA concentration was climbed to 312.73 ~ 349.60 mg/L, much higher than that of reactor for WAS alone (163.98 mg/L), because the HC could enhance the acidogenesis bacteria

activity (Wang et al., 2017). The large promotion of acetic acid in HC-supplemented groups was the reason for enhancement of total SCFA yield. High level SCFA would lead to the pH drop below to the tolerance levels of methanogens and bring negative influence to the methanogenic activities (Li et al., 2015). Thus the methane productions of HC180, HC240 and HC300 in the first 6 days were lower than Blank group (Fig. 1A). At day 8, the propionate showed an absolute predominance in HC-supplemented groups. For example, the level of propionate of HC180, HC240, HC300 was 94.71, 114.33 and 87.87 mg/L, respectively, took the largest proportion between the total SCFA. However, the propionate concentrations of Blank, PC500 and PC700 were close to undetectable levels, and the propionate in PC300 group was at a low level (only 36.93 mg/L). All the results above demonstrated that sludge-based HCs could firstly enhance the production of acetic acid and then propionate, and the PCs (especially PC500 and PC700) have little influence on SCFA composition.

The improvement of SCFA production by HCs could be resulted from the following aspects: (1) Some substances (e.g. humic substance) are aboundant in sludge-derived HCs (Wilen et al., 2003), they act as electron acceptors in the anaerobic progress, then promote the yields of SCFA (Liu et al., 2015). (2) Higher surface area and pore volume were observed in HCs compared with PCs (Table 1). Sludge-derived HCs provide a suitable habitat for acid-producing bacteria, enhance the activity of them.

3.5. Effect of sludge-derived biochars on chemical characteristics of suspension

As shown in Fig. 3A and B, when biochars were added to the WAS digestion system, the pH decreased gradually in the first 8 days, then the pH in all groups kept increasing until the end of anaerobic digestion due to the protein ammonification (Fig. 3E and F) and consumption of SCFA (Fig. 1C and D). The pH of group Blank during whole anaerobic process was higher than those added with HCs (the final pH was 7.46 ~ 7.49). Differing from HCs, the presence of PCs alleviate the pH decline. It was supposed that sludge-based HCs enhance the acidification of hydrolyzed products of WAS (Wang et al., 2017), and PCs maintain more alkaline surface substance then show better buffering capacity than HCs (Yu et al., 2016).

Due to the buffering effect of ammonium and biological inhibition of free ammonia, total ammonia nitrogen (TAN) become a critical role in the performance and stability of digestion of nitrogen-rich substrate (e.g. manure, slaughterhouse waste, WAS). The trend of TAN concentration determined in all mixed liquid samples over time are shown in Fig. 3E and F. The increase of TAN concentration was ascribed to the ammonification of protein and other nitrogen-contained substance. The inhibition from excess ammonia is a concerning issue for a stable anaerobic process of WAS (Rajagopal et al., 2013), but the NH_3 inhibition was not found in this whole anaerobic process. TAN concentrations in the reactors with HCs (especially HC180 and HC240) addition was higher than that of Blank (final TAN concentration follow the sequence HC180 > HC240 > HC300 > Blank). This could be explained by the acceleration of HCs (especially HC180 and HC240) to proteolysis, the presence of HCs resulted in a higher degradation level of nitrogenous organic compounds in WAS. With respect to PCs, TAN of PC500 and PC700 showed a lower level than Blank. This result is consistent with the low soluble organic matters level (especially for soluble protein) in group PC500 and PC700 (Fig. 2). It could assume that PCs produced at high temperature (> 500 °C) have little influence on dissolution and hydrolysis of WAS particle.

The conductivity (EC) of the solution is related to the ion concentration in the solution. EC value of all reactors first increased in the first 20 days, which was caused by the degradation of organic substance, then the EC value slightly decreased (Fig. 3C and D). With biochars addition, the EC value was improved and higher than Blank. During the anaerobic process, the EC of HC300 and PC700 was similar to the value of Blank. And, no matter it is PC or HC, the decrease of pyrolysis or hydrothermal temperature would increase the EC level in liquid phase. It was indicated that the stimulation of biochars to degradation of organic substance is more significant at lower produced temperature. Noticeably, the PC300 showed the highest EC value in all groups. This is may because of the release of the alkalinity substance (i.e. organic anion) retained on the PC300 surface and its relatively low adsorption ability (compared to HCs) to ammonium (Liu et al., 2010). Therefore, the highest EC was attributed to the degradation of substance (Fig. 2), high ammonium (Fig. 3) and the dissolution of organic anion from PC300.

3.6. Effect of sludge-derived biochars on the microbial community

3.6.1. Bacterial and archaeal community composition

The structure and abundance of microbial community are closely related to the presence of biochars in anaerobic reactors. And the microbial communities information was investigated by the Illumina Masiq platform. The number of microbial groups at each classification level and microbial richness between each samples were showed in E-supplementary material. For further investigating, the bacterial and archaeal alpha diversity indexes were exhibited in Table 2. The lower Simpson index and Shannon index of archaea and bacteria in group HCs than those of Blank32d implies that HCs addition decreased the diversity of microbial community (archaea and bacteria). The PCs have little influences on the diversity indexes indicated the PCs have negligible influences on the diversity of microbial community (Table 2).

Principal component analysis (PCA) was performed on the OTUs results. For the bacteria community (Fig. 4A), component 1 and component 2 accounted for 85.29% and 10.32%. The communities of the HCs/PCs-supplemental reactors clustered into a same quadrant except for HC180. For the archaea (Fig. 4B), component 1 and component 2 explained 55.89% and 41.4% of the data variance. When the sludge-derived biochars were added into the WAS reactors, the archaea community showed a significant change. Noticeably, both the biochar-produced temperate and biochar type were responsible for the difference. This results suggested that the HC addition changed the archaea structure significantly but did not change the bacterial community structure largely except for HC180, while PC addition had little effect on the bacterial diversity. In previous study, the surface functional groups of sludge-derived biochars promote the connection of microbes and biochars surface (Wang et al., 2018). Compared to PCs, HCs have more surface functional groups and higher surface area, which could provide more favorable environment for the growth of certain microbe.

Proteobacteria, *Bacteroidetes* and *Chloroflexi* were the predominant phylum in the bacterial communities (E-supplementary data). In the group Blank, the relative abundance of *Proteobacteria* dropped from 35.3% (day 0) to 28.7% (day 32), while it was enriched to 54.0% in HC180. And it still hold a higher proportion of 31.6% and 32.5% in HC240 and HC300 than Blank32d, respectively. However the PCs had not an obvious impact on *Proteobacteria* (28.6 ~ 29.9% proportion on day 32). For *Bacteroidetes*, the relative abundance in HC reactors dropped greater than Blank, and the value was 8.9%, 12.2% and 14.2% in HC180, HC240 and HC300, lower than 16.7% of Blank32d due to the competition among microbes. Differing from HCs, PCs accelerated the enrichment of *Bacteroidetes* (19.2%, 18.2% in PC500 and PC700). These results suggested that HCs provided a favorable environment for the growth of *Proteobacteria*. In genus level, the *Enhydrobacter* and *Candidatus Microthrix* are the major genus in bacterial communities (Fig. 5A). Interestingly, the relative abundance of *Enhydrobacter* even achieved to 37.2% in HC180, however, it was only detected in PC700 and HC180. *Enhydrobacter* is known as sugars fermented facultatively anaerobic bacteria with able to degrade carbohydrate in WAS (Staley et al., 1987). *Candidatus Microthrix was enriched in HC240 and HC300 (3.3 and 3.4%, respectively), while its richness was decreased to 1.8% due to the competition of Enhydrobacter.* The relative abundance of *Candidatus Microthrix*

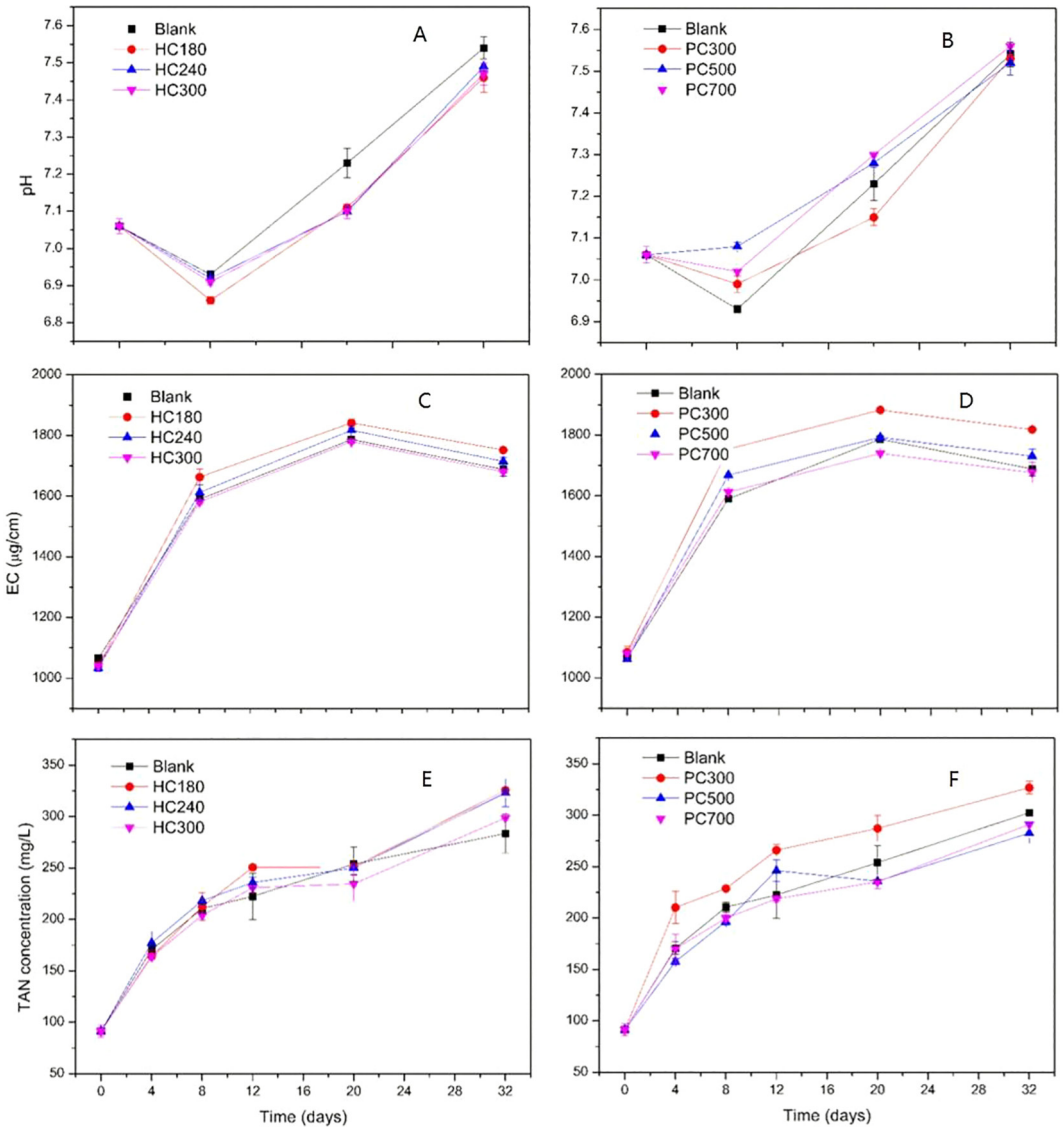

Fig. 3. Variation of pH, TAN and EC during the WAS anaerobic digestion process in presence of hydrochar (A, C, E) and pyrochar (B, D, F).

Table 2
Bacteria and archaea alpha diversity indexes at different sludge samples, and the number of OTU at Phylum level.

Group	Bacteria			Archaea		
	OTU	Simpson	Shannon	OTU	Simpson	Shannon
Blank0d	1313	0.993487	8.63	703	0.900293	5.67
Blank32d	1484	0.991646	8.38	759	0.893367	5.05
PC300	1405	0.991136	8.33	721	0.890140	4.93
PC500	1505	0.991368	8.36	783	0.897495	5.22
PC700	1551	0.991889	8.46	737	0.897620	5.05
HC180	1128	0.860311	6.06	750	0.882731	5.06
HC240	1424	0.989090	8.08	644	0.868076	4.74
HC300	1416	0.989376	8.10	685	0.866285	4.94

in PC300, PC500 and PC700 was 3.3, 2.2 and 2.0%, respectively comparing with that 2.5% of Blank32d. This indicated that higher temperature (> 500 °C) of pyrolysis will inhibit *Candidatus Microthrix*. *Candidatus Microthrix,* a lipid-accumulating and filamentous bacterium, which could assimilate and store the lipid under anaerobic condition (McIlroy et al., 2013). In the recent study, it was reported that strongly positive correlations between the richness of *Candidatus Microthrix* and organic matter removal (Maza-Marquez et al., 2019). Meanwhile, the filamentous structure of *Candidatus Microthrix* has been seen as an inner backbone which is beneficial for the formation of Microbe-EPS aggregation in activated sludge (Maza-Marquez et al., 2019). Therefore, higher hydrolysis acidification rates of HCs were observed than Blank and PCs. And similar result was also obtained in (Wang et al., 2017). Meanwhile, some bacteria communities involved DIET were listed in Table 3. DIET is an important method to exchange electrons between microbes and biochars in the digestion process. And detailed discussion was in Section 3.6.2.

The major archaeal communities at phylum level were *Euryarchaeota, WSA2, Thaumarchaeota, Bathyarchaeota,* which were detected in all groups (E-supplementary data). In Blank, the relative abundance of *Euryarchaeota and Thaumarchaeota* decreased while *WSA2* and *Bathyarchaeota* increased. When HCs were added to the reactors, on day 32, the relative abundance of *Euryarchaeota* in reactors with HC180, HC240 and HC300 presence were still hold higher proportions of 92.6%, 84.5% and 91.3% respectively, while the value in Blank decreased from 96.3% (day 0) to 79.9% (day 32). However, the richness of *Euryarchaeota* in reactors with PCs addition was only between 74%~87.1%. Additionally, the abundance of *Thaumarchaeota* and *Bathyarchaeota* were 0.5% and 0.7% in HC180, 0.5% and 0.6% in

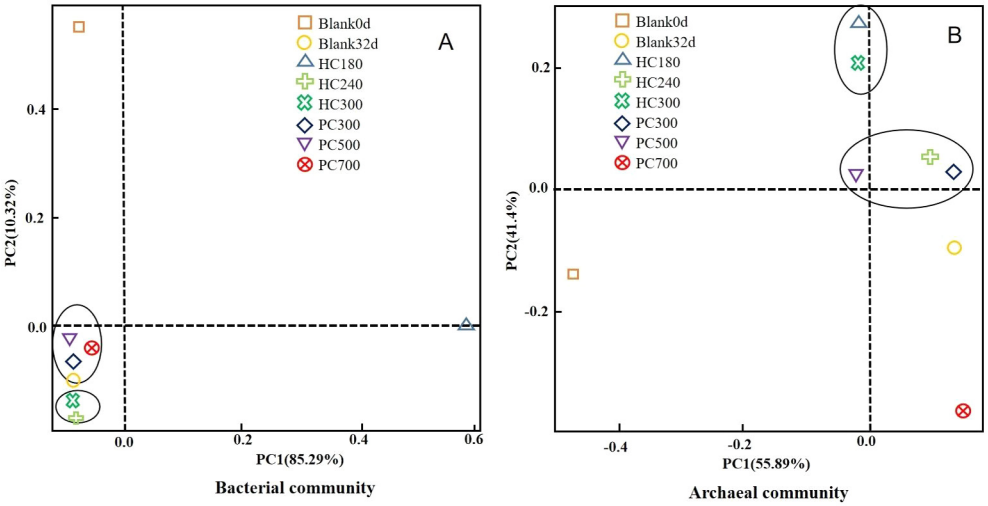

Fig. 4. Principal component analysis of bacterial (A) and archaeal (B) community.

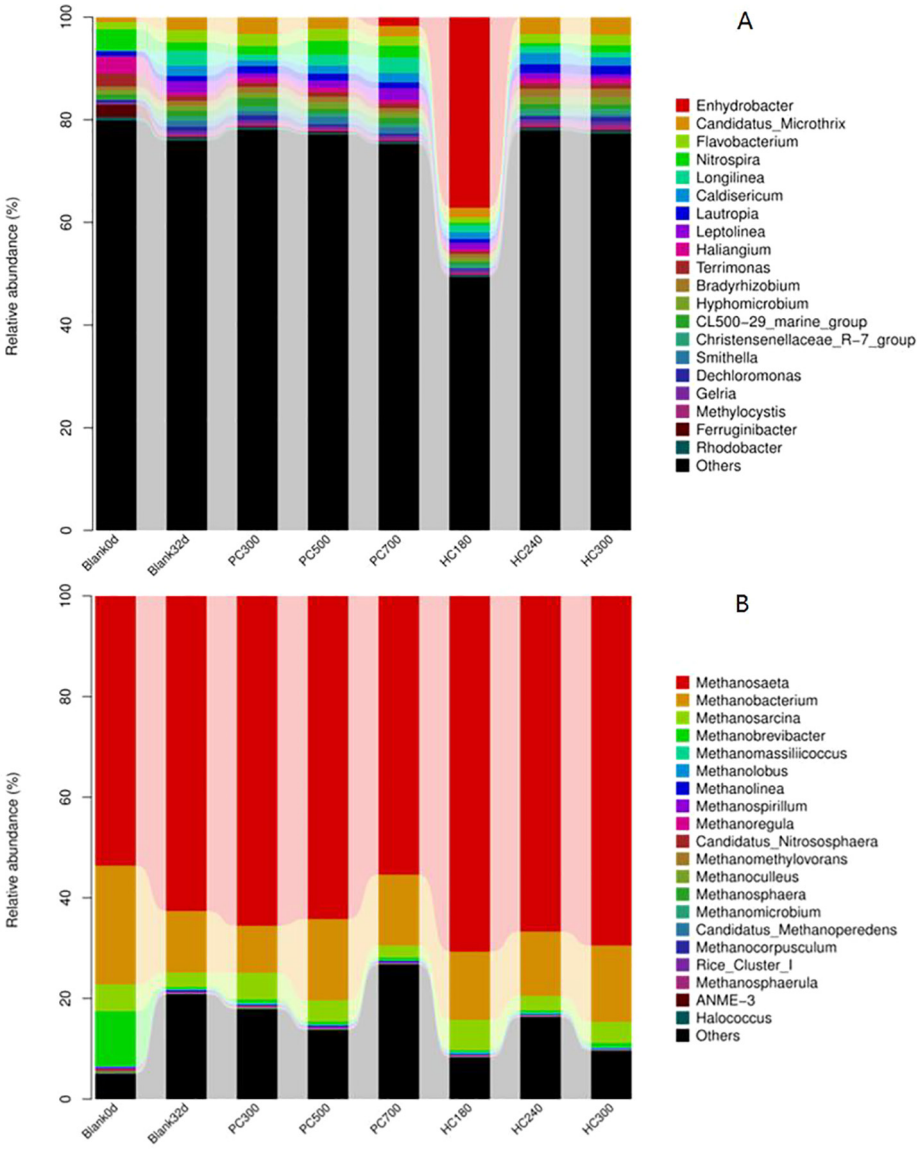

Fig. 5. Relative abundance of bacterial (A) and archaeal (B) community at genus level.

Table 3
Relative abundance of DIET-based microbial community at genus or family level in the reactors with different biochars' addition.

DIET microbes		Blank0d	Blank32d	PC300	PC500	PC700	HC180	HC240	HC300
Bacteria	*Family Peptococcaceae*	0.000%	0.041%	0.111%	0.061%	0.056%	0.035%	0.086%	0.076%
	Genus Syntrophomonas	0.000%	0.137%	0.171%	0.208%	0.222%	0.171%	0.166%	0.186%
	Genus Thauera	0.461%	0.122%	0.151%	0.071%	0.076%	0.106%	0.151%	0.161%
	Genus Geobacter	0.099%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
	Genus Pseudomonas	0.650%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
Archaea	*Genus Methanosaeta*	53.6%	62.6%	65.5%	64.2%	55.4%	70.7%	66.7%	69.5%
	Genus Methanobacterium	23.6%	12.2%	9.38%	16.1%	14.1%	13.5%	12.8%	15.2%
	Genus Methanosarcina	5.35%	2.88%	5.27%	4.21%	2.35%	6.00%	2.85%	4.15%

PC300, which higher than Blank32d (0.2% and 0.5%). That is because the biochars produced at low temperature provide more surface functional groups (E-supplementary data) which are favour in these microbes. At genus level, after 32 days anaerobic digestion, the top 4 aboundant genus in each sample were *Methanosaeta, Methanobacterium, uncultured archaeon* and *Methanosarcina* (Fig. 5B). In previous studies, *Methanosaeta, Methanobacterium,* and *Methanosarcina* had been considered as the archaea with the ability to translate interspecies electrons directly. The relative abundance of DIET archaeal community at genus was showed in Table 3. And detailed discussion of these archaea at genus level was in Section 3.6.2.

3.6.2. Response of DIET-based microbial community to pyrochar and hydrochar

DIET is an important method to exchange electrons in microbes-biochars aggregates. The microorganisms like *Geobacter* directly contact with conductive materials (e.g. activated carbon, biochar) and other microorganisms (e.g. *Methanosaeta*), the electron exchange from *Geobacter* to insoluble electron acceptors or other microbes is achieved via the abiotic conductive materials or the conductive pili (Wang et al., 2018). The enrichment of these DIET-based syntrophic microbes was supposed to be the cause of higher methane production. For better illustrating the enhancement of methane production by biochars, the richness information of bacteria associated with DIET was also showed in the Table 3. Genus *Syntrophomonas*, *Thauera* and Family *Peptococcaceae* which are thought to be involved in the DIET process with methanogens as the partners were detected in our all groups (Zhuang et al., 2015; Zhang and Lu, 2016; Jing et al., 2017). *Geobacter metallireducens*, *Geobacter sulfurreducens* (Lovley, 2017) and *Pseudomonas* (Lin et al., 2017) have been seen as the bacteria that proceed DIET. However, in our studies they were only detected in Blank0d. Interestingly, some studies observed that *Geobacter*- deprived microbial aggregates are capable of performing DIET in methanogenic reactors though conductive mediums such as mineral and graphene materials (Dube and Guiot, 2017; Lin et al., 2018). *Syntrophomonas,* a typical DIET bacteria, was considered to responsible for syntrophic oxidation of butyrate (Zhang and Lu, 2016). The enrichment of *Syntrophomonas* was observed in the reactors, its abundance was between 0.171 ~ 0.186% with HCs addition and 0.171 ~ 0.222% with PCs addition. As the increase of produced temperature, the relative abundance of *Syntrophomonas* is enhanced (except for HC240). That is because that biochars (HC or PC) produced at higher temperature may provide more pore channels as microhabitats for genus *Syntrophomonas.* In our study, the genus *Thauera* also increased from 0.122% (Blank32d) to 0.151%, 0.151% and 0.161% in PC300, HC240, HC300 respectively. This genus was proved able to metabolite propionate and acetate simultaneously (Ma et al., 2015). In a previous study, as magnetite was added to the AD reactor, *Thauera* were found to be enriched and the methane production was stimulated and it was supposed to ascribe to the promotion of DIET capability (Jing et al., 2017). *Peptococcaceae,* a bacterial family was presented that could participate the DIET- mediated syntrophic process with genus *Methanobacterium* (Zhuang et al., 2015). It was enriched in the HC and PC reactors, which was increased from 0.041% (Blank32d) to 0.056 ~ 0.111% in group PCs and 0.076 ~ 0.086% in group HCs (except for HC180).

The relative abundance of *Methanosaeta* and *Methanobacterium* in HC groups were significantly higher than blank32d. Especially for *Methanosaeta*, whose proportion in HC180, HC240 and HC300 was 70.7%, 66.7% and 69.5%, separately, while the WAS digestion without biochars could only achieve a 62.6% relative abundance of *Methanosaeta* on day 32 (Fig. 5B). However, there was not a significant enrichment of *Methanosaeta* in each PC group (55.4 ~ 65.5% relative abundance), and the abundance in PC700 even decrease to 55.4%. In that methanogenic aggregates, *Methanosaeta* even comprised over 90% of methanogens (Morita et al., 2011). The acetate-utilizing *Methanosaeta* could form a DIET-mediated structure with *Geobacter* through the conductive pili (Rotaru et al., 2014). The Fig. 5B indicated that promotion of HCs to *Methanosaeta* is more remarkable than PCs. This was ascribed to that *Methanosaeta* tend to attach the outer surface of biochars, and the more aboundant surface functional groups were provided by HCs (Luo et al., 2015). The addition of biochars (HCs/PCs) alleviated the decline of relative abundance of genus *Methanobacterium* (12.8% ~16.1%) except for group PC300 (9.4%), while the proportion is 12.2% in Blank32d. *Methanobacterium* are recognized as hydrogenotrophic and DIET methanogens, in recently, they were found to be massively enriched in ethanol-methane anaerobic conversion process with conductive graphene addition (Lin et al., 2018). Interestingly, *Methanosarcina*, a methanogen produced methane through various metabolically paths (e.g. aceticlastic, hydrogenotrophic and DIET paths) (Kurade et al., 2019), was only enriched in HC180 from 5.4% to 6% on relative abundance, while the decreases were observed in other groups. Microbial community analysis indicated that sludge-derived biochars especially for HCs facilitate the methanogenic evolution and provide a suitable habitat for specific microbes. And the enrichment of DIET-based syntrophic microbes accelerated the SCFA consumption and methane yields.

3.7. Mechanism of sludge-derived hydrochars to facilitate AD

Compared with sludge-derived PCs, sludge-derived HCs showed positive impact on methane yield from AD of WAS. The precursor WAS mainly consists of flocs through the jointing of extracellular polymeric substance (EPS), thus sludge-derived HCs in this study had larger specific surface area, total pore volume and pore size than PCs (Table 1). Simultaneously, hydrothermal carbonization retained most of surface functional groups of raw material, so HCs had a relatively high level DOC. Previous researches have proven that biochar properties are important for the enhancement of AD. On one hand, due to the good porosity, high surface functionality and ion-exchange capacity of hydrochars, their supplement mitigate the ammonia inhibition of digestion. Meanwhile, there are numerous organic substances (e.g. humic substance) in sludge-derived HCs, they played a role of electron acceptor in AD process, thus SCFA and methane production were promoted (Wilen et al., 2003; Liu et al., 2015). Our previous study found that HCs led to higher hydrolysis acidification rates of WAS than PCs (Wang et al., 2017). On the other hand, the high surface area and

surface functional group of sludge-derived hydrochars provided a suitable habitat for special microorganism and promoted the connection of microbes and biochars surface (Wang et al., 2018). Furthermore, it cannot be ignored that the addition of HCs caused the enrichment of the DIET-based syntrophic microbes such as *Syntrophomonas, Peptococcaceae, Methanosaeta* and *Methanobacterium* (Table 3), which also enhanced the SCFA consumption and methane production.

4. Conclusion

This study demonstrated that sludge-derived HC can better promote the methane production from AD of WAS compared with sludge-derived PC. The highest accumulative methane yield was achieved by HC180, which was 1.2-folds of the Blank. Conversely, PC had a negligible effect on biogas production. Furthermore, HC accelerated the hydrolysis acidification of WAS and had a positive influence on the enrichment of specific syntrophic microbes which participated DIET such as *Syntrophomonas, Peptococcaceae, Methanosaeta* and *Methanobacterium*. Further works in the future should focus on the optimum preparation and modification conditions of sludge-derived hydrochar to achieve more effective AD of WAS.

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.

Acknowledgement

This research was financially supported by National Natural Science Foundation of China (NSFC) (Nos. 51779088, 51709104).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.122235.

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