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Hydrochar promoted anaerobic digestion of hydrothermal liquefaction wastewater: Focusing on the organic degradation and microbial community

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Abstract

Hydrothermal liquefaction wastewater (HTLWW) can be used for biogas production through anaerobic digestion (AD). Nevertheless, it generally contains refractory and toxic organics which causes low methane production. The present study showed that the methane yield and methane production rate were increased by 32–52 % and by 51–84 %, respectively, with the addition of hydrochars derived from different feedstocks including sludge, corn straw, poplar wood and *Enteromorpha* algae. Although hydrochars could adsorb certain amount of organics, the adsorption capacity was not related with the promotion of AD. The adsorbed organics could be neglected after AD due to the biodegradation. 3-dimensional fluorescence excitation emission matrix analysis revealed that hydrochars increased the degradation of all types of fluorescent compounds. Fourier-transform infrared spectroscopy analysis showed hydrochars significantly reduced the concentrations of aromatic, amino acids, amide I and amide II functional groups compounds in HTLWW during AD. Hydrochars also led to higher degradation of nitrogenous, aromatic and phenolic compounds as revealed by gas chromatography mass spectrometry analysis. In addition, it was shown that hydrochars resulted in low number of organic species whose concentration increased during partly degradation. Microbial analysis showed hydrochars enhanced the growth of syntrophic bacteria (e.g. *Syntrophomonas* and *Syntrophorhabdus*) which had ability to degrade an extensive range of various types of organics including aromatic and phenolic organics. Hydrochars also facilitated direct interspecies electron transfer considering the enrichment of known microbes involved in direct interspecies electron transfer (DIET) process including *Geobacter* and certain methanogens (*Methanosaeta* and *Methanobacterium*).

1. Introduction

In China, more than 8 million tons/year dewatered sewage sludge

(DSS) are produced from 4000 domestic municipal wastewater treatment plant (WWTP) as the solid by-product and its disposal has become a crucial environmental issue [1,2]. On the contrary, DSS are rich in

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organics and nutrients, which could be further utilized [3]. Therefore, various thermal, biological and chemical techniques have been applied for the utilization of DSS.

Hydrothermal liquefaction (HTL) is a wet thermochemical process that could produce bio-oil and hydrochar from DSS, and there are extensive studies in recent years [4]. In HTL process, around 20–40% organics and 60–80% nutrients from the biomass are transferred into the aqueous phase, which is called hydrothermal liquefaction wastewater (HTLWW) [2,4,5]. The composition of HTLWW is fairly complicated, and it contains different polymers and toxic organics like alcohols, phenols, benzene, saccharides and variety of nitrogenous heterocyclic compounds, which is dependent on the reaction conditions [2,4,6,7]. Discharge of HTLWW without pre-treatment would be a waste of resources and lead to serious environmental damages. Therefore, it is very important to recover the energy and reduce the pollutants from HTLWW before discharging into the environment.

Anaerobic digestion (AD) is a promising technique which has been applied for the degradation of organics to produce energy from HTLWW in the form of biogas in recent years [2,4,8–10]. Nevertheless, HTLWW has low conversion efficiency in AD due to complex in nature [6,9]. For example, it was reported that the methane yield was below 200 mL/g COD for HTLWW obtained from HTL of sewage sludge at temperature higher than 260 °C, which was the normal condition for bio-oil production. In above study, the obtained methane yield was lower than 350 mL/g COD (theoretic value) [2,9]. Therefore, the organic conversion efficiency of AD should be increased to make the whole process more economical and feasible.

Previous studies applied several techniques to improve the biodegradation of the organics and remove the toxic organics in HTLWW. For example, the methane yield was increased by 109% from HTLWW obtained from HTL of swine manure through ozone pre-treatment, which increased the degradation of the toxic and resistant organics [11]. The removal of toxic and resistant organics via solvent extraction (organics) from rice straw HTLWW increased the methane yield by 28% [9]. In addition, the biodegradation of HTLWW through AD could also be increased by using different adsorbents (E.g., granular activated carbon (GAC), polyurethane matrices and zeolite) [8] and powder activated carbon [12]. For instance, GAC, which has high adsorption capacity for organics, was used in AD to enhance the methane yield. It was noticed the methane yield was increased by 28–67 % from different HTLWW with the addition of GAC [2,8,13]. The previous studies mainly thought GAC could adsorb toxic organics in HTLWW, and therefore, resulted in increased methane yield. Recently, it has been found that GAC could also promote syntrophic growth of microbes to degrade more organics for enhancement of methane production [2]. It seems both adsorption and enrichment of syntrophic microbes contributed to the enhanced AD of HTLWW by GAC [2,11,14,15]. It should be noted GAC is a relatively expensive material and it is necessary to develop more cheap materials to enhance the methane production from HTLWW.

Hydrochar is a carbonaceous solid product with rich surface functional groups generated from HTL process, and has been used for different applications such as soil amendments, and solid fuels [4,16,17]. The enhancement of methane production by using hydrochar has been sporadically testified [1,18,19]. A previous study suggested that the addition of hydrochar in AD decreased the concentration of free ammonia, hold nutrients, and slightly decreased pH, which improved the methane yield and production rate from dead pig carcass [20]. It showed that hydrochar had potential to be utilized in AD process. However, it is still unknown whether hydrochar could promote methane production from HTLWW, considering the high contents of toxic and refractory organics. Hydrochar can be prepared from various feedstocks, which might also affect the characters (e.g. BET surface area and functional groups) of hydrochar. Different hydrochars might have different effects on AD process, which deserved to be investigated.

By considerations of above aspects, the current study intended to explore the AD performance of HTLWW with hydrochar derived from

different feedstocks, and it was also compared with GAC. In addition, considering the presence of complex organics including both low and high molecular weight organics in HTLWW, different analytical tools including gas chromatography–mass spectrometry (GC–MS), 3-dimensional fluorescence excitation emission matrix (3D-EEM) spectroscopy and fourier-transform infrared spectroscopy (FTIR) were applied to better understand the overall characters of HTLWW before and after AD [4]. Moreover, the changes of microbial communities due to the addition of hydrochar and GAC were also analysed by high-throughput sequencing of 16S rRNA genes.

2. Material & methods

2.1. Preparation of hydrochars

Hydrochars were obtained by HTL of different feedstocks. Sludge (SL) was obtained from wastewater treatment plant, Shanghai, China. Corn straw (CS), poplar wood (PO) and *Enteromorpha* algae (EN) were also used as feedstocks. HTL was operated in a 3 L hydrothermal reactor by keeping common condition for bio-oil production [4]. Each time, biomass was encumbered in the reactor and the same procedure as described in our previous study was followed [2,21]. The attached bio-oil on hydrochars were removed with tetrahydrofuran, and the hydrochars were then dried for further utilization. In addition, granular activated carbon (GAC) was procured from local market and used in order to make comparison with hydrochars. All types of hydrochars and GAC were grounded and sieved (2 mm) before usage. The physical and chemical properties of hydrochars and GAC were described in Table 15.

2.2. HTLWW from DSS

Dewatered sewage sludge (DSS) was also obtained from same treatment plant as mentioned above with the moisture content of DSS was 83.9%. HTLWW was produced by loading 2 L DSS in the same 3 L hydrothermal reactor as used for hydrochars preparation and kept similar reaction conditions. The liquid and solid products were obtained by centrifuged at 5000 rpm for 2 min and then HTLWW liquid was stored at –20 °C for further usage. The HTLWW characteristics are shown in Table 25.

2.3. Biogas production potential tests

The methane production potentials of HTLWW were determined in batch mode by using 118 mL serum bottles with the addition of different carbon materials (hydrochars and GAC). The inoculum was obtained from AD reactor treating cassava stillage, and the characteristics are as follows: pH = 7.3 ± 0.2, TS = 36.9 ± 1.4 g/L and VS = 29.9 ± 0.9 g/L. In each bottle, the required amount of HTLWW and water, different materials (hydrochars and GAC), and 2 mL inoculum were added to attain the final working volume 60 mL, COD 6 g/L, and materials 10 g/L (hydrochars and GAC). The bottles containing only inoculum were mentioned as blank, and the bottles without hydrochars and GAC were mentioned as control (C). The solution of sodium hydroxide (2 M) and hydrochloric acid (2 M) were prepared and used to adjust the pH of the bottles to 7.5. The bottles were then placed in an incubator at 37 °C. The temperature was kept constant throughout incubation period and the experiments were done in triplicates.

The Gompertz model (Eq. (1)) was applied to simulate the methane production with different hydrochars and GAC.

$$M(t) = P \times \exp \left\{ -\exp \left[\frac{Rm \times e}{P} \times (\lambda - t) + 1 \right] \right\} \quad (1)$$

where, $M(t)$ is the cumulative methane production (mL) at time t , P is the maximum methane potential (mL methane), Rm is the maximum methane production rate (mL/d), λ is the lag-phase time (d), and e is

2.71828. The parameters, P , R_m , and λ were assessed by using OriginPro 2016 [22].

2.4. Adsorption capacity of hydrochars and GAC

Adsorption experiments were conducted in triplicates to measure the adsorption capacity of inoculum, different hydrochars and GAC towards organics in HTLWW. Two types of kinetic adsorption models including pseudo first and second order models were used for simulation [23,24]. In each bottle, HTLWW (COD 6 g/L), different hydrochars and GAC (10 g/L), were added to attain the same working volume as kept for biogas potential tests, and the bottles were put in a shaker at 150 rpm with constant temperature at 30 °C. Similar experiment was also performed with inoculum (same concentration as used in biogas production potential tests), HTLWW and water. The liquid samples were collected with specific time intervals up to 14 h.

2.5. Microbial community analysis

Microbial samples were collected from all the reactors at maximum methane production. One sample from each reactor was collected, and the samples from the triplicate bottles were mixed together before DNA extraction. The DNA extraction, PCR method and taxonomic classification were conducted by following the described procedures in our previous studies [7,9,25,26].

2.6. Other analytical methods

The chemicals and its compositions in HTLWW were quantified by using GC-MS (Focus DSQ, Termoelectron, America), and followed the previously described procedure [6,9,10].

Fluorescence spectrophotometer (Horiba, Japan) was used to detect the fluorescent components and its composition in HTLWW before and after AD. The detail of the analysis was explained in our previous study [10].

HTLWW and the liquid samples after AD were vacuum dried for 72 h for the detection of functional groups of various compounds by Fourier transform infrared spectroscopy (FTIR) Nexus470 (Thermo Fisher Scientific, USA) with the resolution of 2 cm^{-1} over a range of 400 to 4000.

Gas chromatography (GC 960) was used to determine the methane gas composition by following the previously described procedure [10,27]. Volatile fatty acids (VFAs) composition were measured by GC (Shimadzu, G2010) [9]. pH was detected with pH meter (FE20, Mettler Toledo, Switzerland). Ammonium nitrogen, total nitrogen (TN) and chemical oxygen demand (COD) were determined by following APHA [28]. Total organic carbon (TOC) was measured by TOC analyser (TOC-L CPH, Shimadzu, Japan).

Automated surface and pore size analyzer (Quantachrome, USA) was used to determine Brunauer-Emmett-Teller (BET) surface area of hydrochar. Elemental analysis was conducted by an elemental analyzer (Vario EL cube, Elementar, Germany).

2.7. Data analysis

The linear correlation between the amount of HTLWW adsorbed at equilibrium and methane yield were determined by using Pearson's correlation through SPSS version 20.0 [3,29]. Two-tailed t -test was performed to determine the Pearson's correlation coefficient (R) at p -value < 0.05 [10].

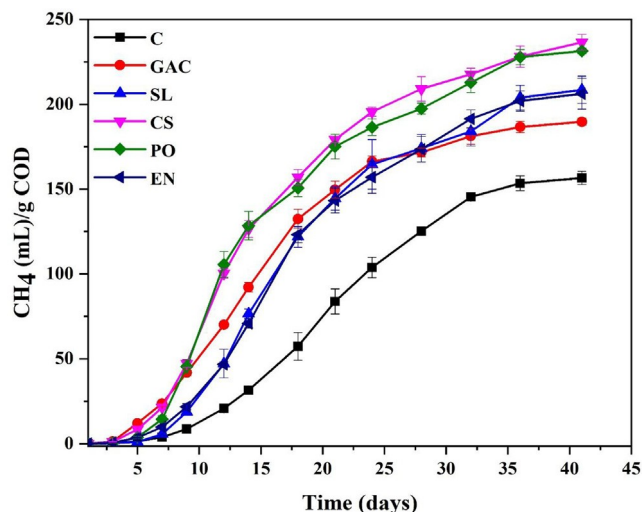


Fig. 1. Cumulative methane production from HTLWW with the addition of hydrochars and GAC.

3. Results & discussions

3.1. Effects of hydrochars and GAC on methane production and organic conversion

The batch experiments were conducted for more than 40 days until the maximum methane yields were achieved. It was noticed that higher methane yields were obtained from the bottles with hydrochars and GAC ($> 190\text{ mL methane/g COD}$) than the C bottles ($156\text{ mL methane/g COD}$). In addition, hydrochars had better capacity to promote methane production from HTLWW as compared to GAC (Fig. 1), and the highest methane yield of 237 mL/g COD was attained from CS reactor. In a previous study, $212\text{ mL methane/g COD}$ was achieved from HTLWW of swine manure by using GAC (20 g/L) which is very close to the present results [11]. The kinetic parameters of methane production obtained by fitting the modified Gompertz equation are shown in Table 1, and it was shown that higher methane yields were also accompanied with higher methane production rates. For instance, it was noticed that CS significantly improved the methane production rate to 13.71 mL/d , which was around 84% higher than that of C reactor (7.44 mL/d). At the same time, CS also impressively affected on lag phase and reduced it to 5.39 d from 9.82 d of C reactor. Although GAC also led to the shorter lag phase (5.40 d), the methane yield (190 mL/g COD) of GAC reactor was lower than that ($> 206\text{ mL/g COD}$) of hydrochar reactors. The above results indicated hydrochars had higher capacity to promote methane production from HTLWW compared to GAC. The parameters of liquid after AD were described in Table 2. The COD removal efficiency of 54% was achieved in C reactor, while the hydrochar reactors had higher COD removal efficiencies (77%–70%). It should be noted GAC reactor had the highest COD removal efficiency of 92% although the methane yield was lower than all the hydrochar

Table 1
Kinetic parameters of the methane production by fitting the modified Gompertz model.

Samples	Measured maximum CH_4 production (mL)	P (mL)	R_m (mL/d)	λ (d)	R^2
C	156 ± 3.95	153	7.44	9.82	0.999
GAC	190 ± 2.05	190	10.89	5.40	0.999
SL	209 ± 8.06	205	11.78	7.86	0.996
CS	237 ± 4.66	229	13.71	5.39	0.995
PO	231 ± 1.77	221	13.72	5.49	0.986
EN	206 ± 9.12	207	11.20	7.62	0.998

Table 2
Characteristics of HTLWW before and after AD with different hydrochars and GAC.

Characteristics	Influent	C	GAC	SL	CS	PO	EN
pH	7.5 ± 0.05	7.09 ± 0.02	7.42 ± 0.04	7.01 ± 0.03	7.16 ± 0.02	7.05 ± 0.03	7.19 ± 0.02
COD (mg/L)	6000 ± 100	2766 ± 80	458 ± 10	1705 ± 40	1361 ± 30	1407 ± 35	1805 ± 20
TOC (mg/L)	2348 ± 150	1093 ± 100	319 ± 20	823 ± 45	708 ± 35	771 ± 20	799 ± 40
TN (mg/L)	657 ± 50	592 ± 15	540 ± 20	498 ± 10	435 ± 17	423 ± 13	508 ± 10
NH ₄ ⁺ -N (mg/L)	483 ± 20	468 ± 15	462 ± 13	413 ± 10	393 ± 18	379 ± 11	421 ± 17
Acetic acid (mg/L)	682 ± 80	2.9 ± 0.1	1.1 ± 0.15	1.02 ± 0.09	1.44 ± 0.13	2.56 ± 0.18	1.01 ± 0.05
Propionic acid (mg/L)	175 ± 30	4.8 ± 0.2	/	/	/	/	1.61 ± 0.15
<i>i</i> -butyric acid (mg/L)	80 ± 10	1.5 ± 0.1	/	/	1.61 ± 0.12	/	1.39 ± 0.05
<i>n</i> -butyric acid (mg/L)	213 ± 50	1.6 ± 0.1	/	/	/	/	/
<i>i</i> -valeric acid (mg/L)	142 ± 20	1.2 ± 0.1	/	/	2.60 ± 0.15	/	1.26 ± 0.08
<i>n</i> -valeric acid (mg/L)	54 ± 10	1.0 ± 0.1	/	/	/	/	/

reactors. It is known GAC had high adsorption capacity and it was possible GAC adsorbed more COD that was not converted to methane, which was discussed in the following part [11].

The pH in all the reactors were varied between 7.09 and 7.42 for the whole incubation period, which was suitable for AD [2,10]. Furthermore, the concentrations of TN and NH₄⁺-N were lower after AD with no significant differences among all the reactors, which might be due to the microbial growth. The concentrations of VFAs in all reactors after AD were very low (< 5 mg/L), which indicated methanogenesis was not the limiting step in all the reactors. The lower COD removal efficiency in C reactor might be due to that some refractory organics were not efficiently acidified to produce VFAs due to the lack of certain microbes.

3.2. COD mass balance and adsorption capacity of hydrochars and GAC

Fig. 2 shows the COD mass balance for the different reactors. For reactor C, since there was no hydrochars and GAC addition, the adsorbed COD could be neglected (the adsorption of organics by inoculum was also negligible). Therefore, the adsorbed and microbial utilized COD (A + M, calculated on the basis of COD mass balance by Eq. (2)) part could be mainly due to microbial growth.

$$A + M = TotalCOD - ResidualCOD - MethaneCOD \quad (2)$$

It was obvious GAC reactor had the highest amount of A + M COD (38%). While all the hydrochar reactors had lower A + M COD (10–12%) as compared with GAC, and no significant difference was observed among A + M COD (9%) of C and hydrochar reactors ($P < 0.05$, ANOVA). Although it was not known the exact amount of COD used for microbial growth in GAC reactor, it could be close to that in C reactor. Therefore, the A + M COD of GAC reactor indicated GAC adsorbed more COD, which was not utilized for methane production.

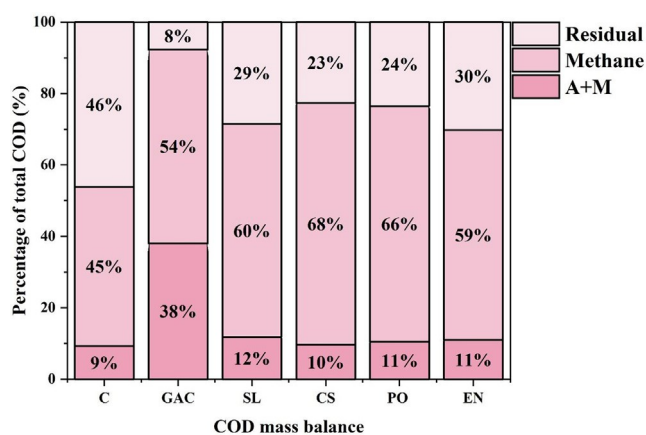


Fig. 2. COD mass balance, and A + M showed adsorbed and microbial utilized COD).

Adsorption experiments were further performed to test the adsorption capacity of different hydrochars and GAC. Pseudo first order and pseudo second order were used for adsorption kinetic analysis [23,30]. Fig. 1S shows, pseudo second order kinetic model fitted better to the adsorption due to high R^2 values (0.9857–0.997). It was noted that GAC had the highest adsorption capacity of 263.16 mg.g⁻¹ for the organics in HTLWW due to high BET (971.33 m²/g) (Table 1S). Although CS had the lowest adsorption capacity of 64.94 mg.g⁻¹ and had lowest BET (63.08 m²/g), it resulted in higher methane yield and higher residual COD (23%) as compared with GAC. Previous studies considered that the adsorption capacity of GAC was important in the enhancement of methane yield of HTLWW [11,13,31]. However, the present study showed adsorption might not be necessarily related with the enhancement of methane yield. For instance, Pearson's correlation coefficient between methane yield and adsorption capacity at equilibrium showed the strong negative correlation (Table 3S). The high adsorption capacity of GAC could result in lower amounts of substrates for methane production since the adsorbed organics might include easily biodegradable organics. It should be noted that although hydrochars had certain capacities to adsorb organics, these organics might be further released into the liquid for utilization since the A + M COD in hydrochar reactors were close to reactor C. Therefore, the organics adsorbed by hydrochars after AD could be neglected. In order to understand the role of hydrochars in enhancement of AD of HTLWW, further investigations including molecular and microbial analysis were conducted.

3.3. Characterization of recalcitrant organics in liquid after AD

Different technologies were used to characterize the organics in the HTLWW and liquid after AD. Considering the neglectable organics adsorption by hydrochars after AD as mentioned previously, the differences between HTLWW and the liquid after AD could be assumed to be mainly due to biodegradation. However, both biodegradation and adsorption contributed to the differences between HTLWW and the liquid after AD for GAC, since it still absorbed considerable organics after AD.

3.3.1. Effect of hydrochars and GAC on the degradation of fluorescent components

The fluorescent components in HTLWW samples before and after AD were analysed by 3D-EEM (Fig. 3). Four components (C₁–C₄) were identified by PARAFAC analysis (Fig. 2S). C₁ had one Ex and one Em peak at 287/388 and it was related with microbial by-product substance. C₂ had two Ex and one Em peaks at 248, and 318/400, which were associated with fulvic acid-like substances. C₃ had only one Ex/Em peak at 280/411, and it was linked with humic and fulvic-like substances. C₄ had two Ex/Em peaks with the first peak at 270/300 and second at 357/435, and these were affiliated with humic acid-like substances [10,32–34]. C₁ was dominant in raw HTLWW, and it was described as biodegradable organics (Fig. 4) [2,35]. While the components C₂, C₃ and C₄ belonged to the humic and fulvic acid-like substances, which could only be degraded to a certain level [2,35].

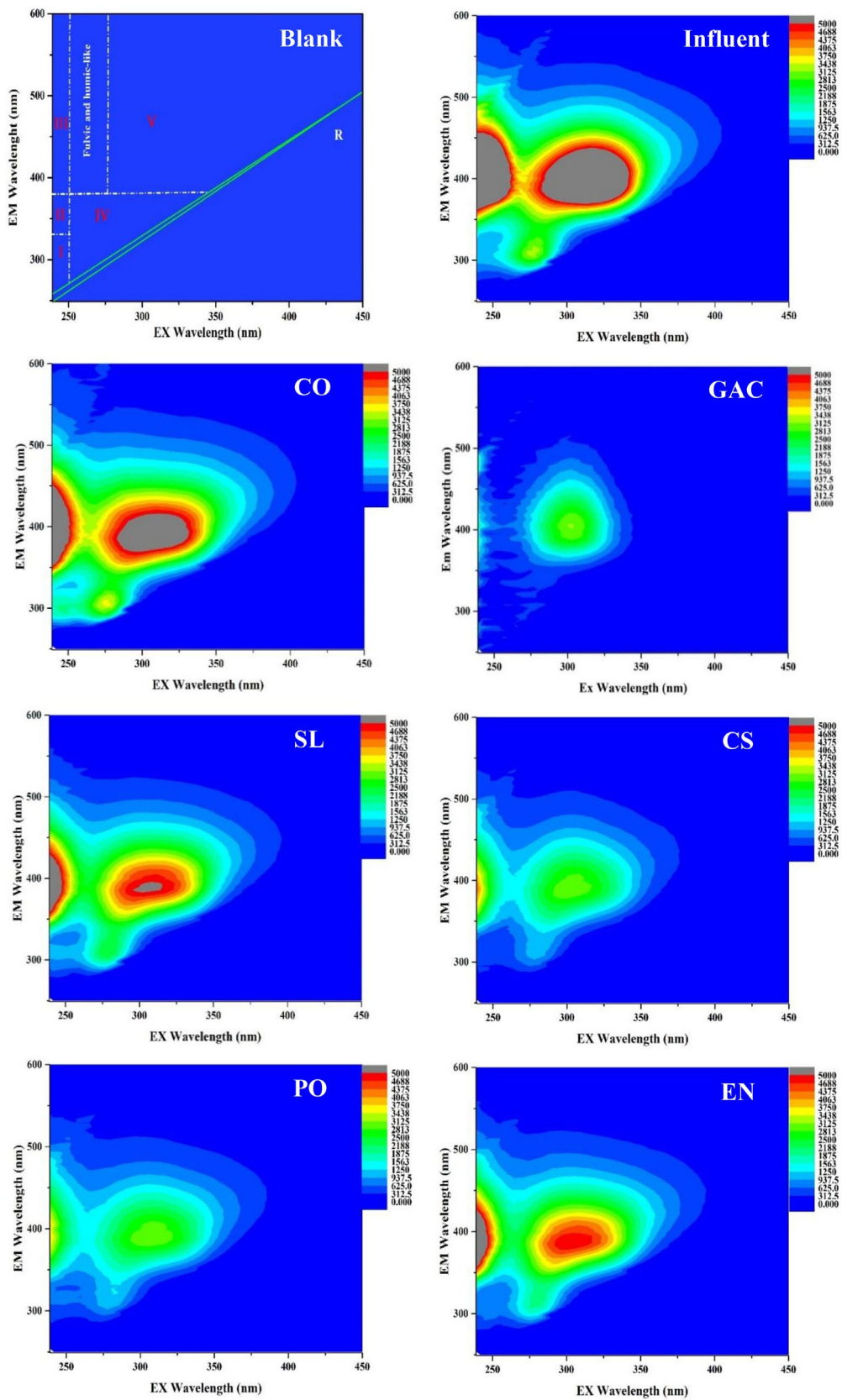


Fig. 3. EEM analysis of HTLWW samples at maximum methane yields.

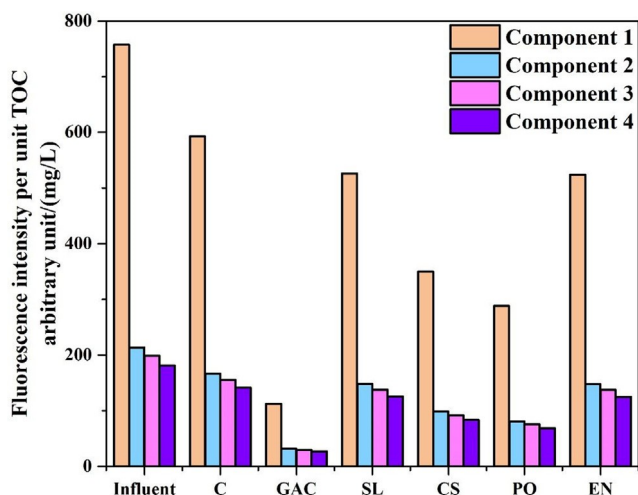


Fig. 4. Fluorescence intensity of the EEM-PARAFAC C₁-C₄ components in different samples.

Furthermore, it was observed the concentrations of components C₁-C₄ were decreased in all the reactors, and the addition of hydrochars promoted the degradation of such components in HTLWW, which could be due to the formation of different microbial communities among all the reactors. It should also be noted that the lowest concentration of C₁ in HTLWW was observed in PO and CS reactors. Considering the low adsorption capacity, it suggested C₁ was well degraded in these two reactors. Although GAC resulted in the lowest concentrations of fluorescent components C₁-C₄ especially C₁ (degradable component), it might be adsorbed due to high adsorption capacity instead of degradation, which could be reflected by the lower methane yield as compared with that of hydrochar reactors (Fig. 4). These results were consistent with COD mass balance and achieved low residual COD as compared with hydrochars. The above results presented the fluorescent compounds were well degraded during AD with the addition of hydrochars, and thus resulted in higher methane yield.

3.3.2. Effects of hydrochars and GAC on the functional groups of HTLWW

The FTIR was performed in the region from 400 to 4000 cm⁻¹ to identify the changes of functional groups of HTLWW during AD. Table 4S showed that the functional groups were divided into five categories including hydrocarbons, protein, carboxylic group-containing

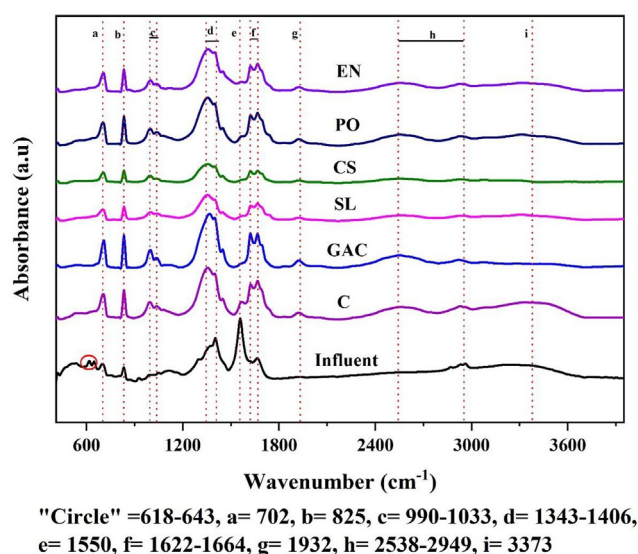


Fig. 5. FTIR analysis of HTLWW before and after AD.

& hydrocarbon-like compounds, polysaccharides & nucleic acids, and finger print region [20,36,37]. As shown in Fig. 5, the region from 600 to 3600 cm⁻¹ provided the main information regarding the composition and functionality of HTLWW. It was noticed that the finger print region (600-900 cm⁻¹) was greatly affected by AD process. The raw HTLWW had obvious peaks at the region from 618 to 643 cm⁻¹, associated with amino acids which completely disappeared due to well degradation during AD. While the peaks at region 702 cm⁻¹ and 825 cm⁻¹ appeared in large size after fermentation, which belongs to C-H deformation, further associated with branched and aromatic hydrocarbons [38]. On the other hand, obvious peaks appeared after AD in a region from 990 to 1033 cm⁻¹ due to the presence of alcoholic and phenolic compounds in high concentrations [20,39]. Furthermore, the peaks in raw HTLWW were reduced by AD at the regions from 1343 to 1406 cm⁻¹, 1550 cm⁻¹ and 1622-1664 cm⁻¹, and associated with amide I and amide II, which might belong to the carboxyl and partly aldehyde, ketone, and ester compounds [20,38].

Moreover, it was noticed hydrochars promoted the removal of aromatic amino acids, C-H, C-OH, and C-O functional groups in the regions 702, 825 and 990-1033 cm⁻¹, and resulted in smaller peaks as compared with C. It should also be noted all the hydrochars had smaller peaks except PO as compared with C, indicating more organics were degraded and more substrates were produced for methane production. Furthermore, it was observed CS had high removal efficiency of aromatic, nitrogenous and nucleic acids compounds, and resulted in higher methane yield. However, PO reduced the concentration of aldehyde and ketonic compounds instead of amide I & II functional group compounds, and it might be due to different structural properties (e.g. functional groups) of hydrochar (PO) as compared with CS [40]. In short, all the hydrochars significantly reduced the concentration of aromatic, amino acids, amide I and amide II functional groups compounds in HTLWW as compared with C, which resulted in the higher methane yields.

3.3.3. Effects of hydrochars and GAC on organic composition of HTLWW

The chemical species in the HTLWW samples were revealed by GC-MS analysis. 42 organics were found in raw HTLWW and listed in Table 5S, while 11 organics completely disappeared after AD, which were assumed to be biodegradable organics and therefore not found in all reactors. The organics were categorized into five major divisions, and showed on the basis of absolute peak area by GC-MS analysis in Fig. 6 [2].

Nitrogenous organics were dominant in raw HTLWW, which might be due to the degradation of protein at high reaction temperature during HTL of DSS [2,4,7], while aldehyde and ketone compounds were the second major organics in raw HTLWW. However, higher disappearance of nitrogenous compounds was observed after AD, while no significant difference was noticed in class of aldehyde and ketone compounds except SL reactor. It should be noted that nitrogenous

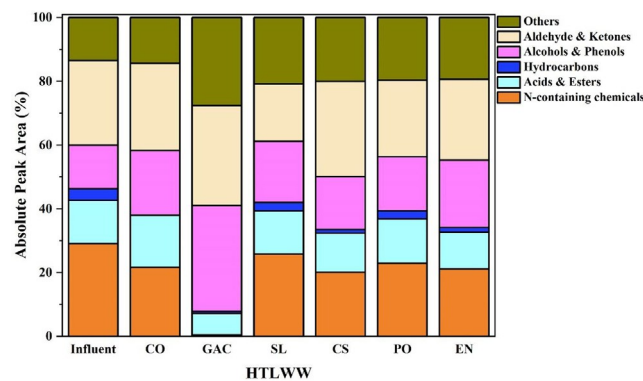


Fig. 6. Organic compositions in HTLWW by GC-MS analysis.

species was not found in GAC reactor, which could be due to the higher adsorption capacity of GAC compared with hydrochars. Table 6S showed, 31 organics disappeared in all the reactors with different removal efficiencies while pyrazine, 2-ethyl-5-methyl-, 1-butanamine, N-propyl- and 9-octadecen-1-ol, (Z)- were well degraded in all the reactors. Furthermore, it was also noticed that the concentrations of some organics were increased (on the basis of absolute peak area). The concentrations of 9, 3, 7, 4, 4, and 7 organic species increased in C, GAC, SL, CS, PO and EN reactors, respectively, which might be due to partly degradation or restructuring of other organics (Table 6S). For example, pyrazine, 2-ethyl-3-methyl-pyrazine has similar structure like 2-ethyl-5-methyl-, and its concentration was increased in SL reactor during AD, which might be due to restructuring of the organic. On the other hand, the concentration of oxacyclododecan-2-one was decreased while the concentration of undecanolactone was increased, which might be due to partly degradation in SL reactor. However, least organics species were present in CS and PO reactors as compared with C and other hydrochar reactors excluding GAC, which might be associated with the better degradation of organics due to the enrichment of certain microbes, and the results were consisted with EEM and FTIR results (Figs. 4 and 5) [2]. In addition, the similar behaviour of CS and PO towards organics degradation and methane production could be due to same source of biomass for hydrochar production (lignocellulosic material) [40].

Few organic species were not found in the raw HTLWW but detected in the residual HTLWW, and the newly produced chemical organics were listed in Table 6S. There were 9, 8, 9, 10, 12, and 10 kinds of new organic species appearing in C, GAC, SL, CS, PO and EN reactors, respectively, along with 17 types of shared organics. These new organics might be originated as mediators from the degradation of branched chain and aromatic structure organics, which were present in the raw HTLWW and transformed into hydrocarbons (straight & short chain) by dehydrogenation and deoxygenation in AD. GC-MS results were consisted with FTIR analysis (Fig. 5). For example, naphthalene 1-(1-methylethyl)-, 2-ethoxyethyl acrylate, benzene, 1,2-(methylenedioxy)-4-propenyl- (E)-, phenol and γ -dodecalactone were found in the raw HTLWW with larger peaks (absolute area), while these organics were completely disappeared and could be transformed into hexadecane, (S)-(+)-1,2-propanediol, n-hexadecanoic acid, 2-heptanol 3-methyl- and propane 2-methoxy-2-methyl- with larger peaks after AD (Table 6S). The subordinate residual aromatic organics intensity could result in high methane yield since aromatic chemicals were recalcitrant organics for AD [4,6,41]. Based on the above analysis, it was suggested that hydrochars enhanced the degradation of aromatic and phenolic compounds, and resulted in higher methane yield as compared with C (Table 5S).

3.4. Effects of hydrochars and GAC on microbial communities

The microbial communities were further analysed and PCA analysis showed the samples with hydrochars and C were well separated from the sample with GAC, indicating the unique microbial community formed with GAC. It should also be noted the microbial communities had certain dissimilarity between the samples with hydrochars since they were not overlapped in Fig. 3S.

The taxonomic classifications of the sequences at phylum level are shown in Fig. 7a. *Proteobacteria*, *Firmicutes*, *Acidobacteria*, *Bacteroidetes* and *Chloroflexi* were dominant phyla in all the reactors. All types of hydrochars significantly increased the relative abundance of *Bacteroidetes* to higher than 12%, which was only 10% in C reactor. *Bacteroidetes* contains acidogenic bacteria, which could produce VFAs from glucose and aromatic organics [33,42]. Previous studies also reported *Bacteroidetes* was the main phyla in AD reactors treating the phenolic wastewater [43,44]. Therefore, its enrichment with hydrochars might relate with the higher methane yields due to the degradation of more aromatic and phenolic organics, which were also demonstrated by

GC-MS.

Fig. 7b shows the changes of relative abundances of bacteria at the genus level. The relative abundances of *Syntrophorhabdus*, *Syntrophomonas*, *Gracilibacter* and *Geobacter* were increased with the addition of hydrochars during AD. Although all hydrochars promoted biogas production, the enriched microbes were different. It was noticed higher relative abundances of *Syntrophorhabdus* (11–12%) and *Syntrophomonas* (10–11%) were enriched with the addition of hydrochars as compared with reactor C (8% and 7%, respectively). The two microbes are known as syntrophic bacteria which could be involved in interspecies H₂ transfer (IHT). *Syntrophorhabdus* has the ability to degrade phenolic compounds into acetate with hydrogenotrophic methanogens, and might result in higher methane yield in hydrochar reactors [2,45]. This behaviour was observed in GC-MS analysis in Table 5S. *Syntrophomonas* could consume the long and short chain fatty acids with hydrogenotrophic methanogens. Further, the growth of *Syntrophomonas* in co-culture could also oxidize CH₃COO⁻ and C₃H₅O₂⁻ by using protons as the electron acceptor with hydrogenotrophic methanogens [2,46]. Furthermore, the relative abundance of *Gracilibacter* was increased to 2–6% in hydrochar reactors while it was not found in reactor C. *Gracilibacter* was reported to produce H₂ and short chain fatty acids from protein and carbohydrates at acidogenesis stage and these acids can be further consumed by syntrophic bacteria (above mentioned) to produce CH₃COO⁻ and H₂ [2,47]. In addition, *Geobacter* was also not found in C reactor, while its relative abundance was increased to 2% in reactors SL and EN. It was noteworthy that *Geobacter* was obviously enriched in reactors CS and PO with the relative abundance of around 7% for both reactors. *Geobacter* is generally known as electroactive bacteria participating in direct interspecies electron transfer (DIET), which could promote methane production rate from organics, and its enrichment indicated hydrochars facilitated DIET during AD of HTLWW [31,48,49].

Fig. 7c shows the significant differences in the relative abundances of methanogenic archaea at the genus level. The relative abundance of *Methanobacterium* was 47% in C, while it was decreased (32–43%) with the addition of hydrochars, and it is hydrogenotrophic methanogens, which produces methane from H₂ and CO₂ [33,50]. The relative abundances of *Methanoseta* (26–34%) and *Methanolinea* (11–16%) were increased with the addition of hydrochars as compared with C reactor (23% and 5% respectively). *Methanoseta* (known as *Methanotrix*) is an acetoclastic methanogens, and it could also participate in DIET for methane production by reducing CO₂ [2,51]. In this study, it was noticed DIET process could be performed between archaea genus such as *Methanoseta* & *Methanobacterium* and bacteria genus such as enrichment of *Geobacter* & *Syntrophorhabdus*, and resulted in higher methane production rate compared with that of C reactor [52–54]. *Methanolinea* is a hydrogenotrophic methanogen and it could be associated with the growth of syntrophic bacteria for conversion of H₂ and CO₂. Furthermore, it should also be noted that the addition of CS and PO increased the relative abundance of *Methanomethylovorans* to 5% and 4%, as compared with C (< 0%), respectively. The relative abundance of *Methanosarcina*, which could mediate both hydrogenotrophic and acetoclastic methanogenesis, decreased to 7% and 6% with the addition of CS and PO compared with C reactor (17%), respectively.

3.5. Implications

The present study made the first attempt to apply hydrochars in AD of HTLWW. It showed hydrochars increased the methane production from HTLWW, and it was mainly related with the growth of specific microbes (e.g. *Bacteroidetes* and *Syntrophorhabdus*), which were reported to have the ability to degrade nitrogenous and phenolic organics as confessed by FTIR and GC-MS analysis [52]. In addition, hydrochars also enhanced the methane production rate, which could be related with its ability to facilitate the formation of DIET in AD process as reflected by the enrichment of certain known bacteria involved in DIET

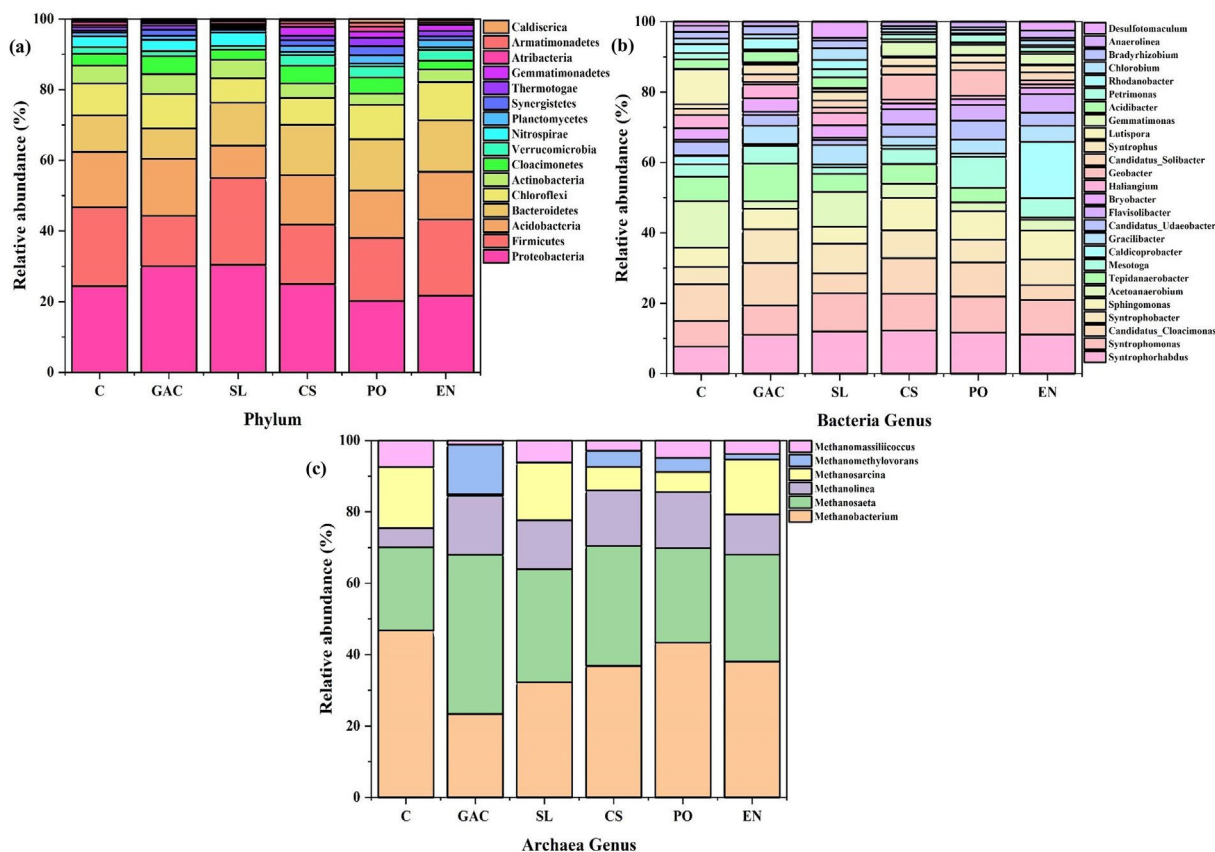


Fig. 7. Microbial community compositions at (a) phylum level, (b) bacterial genus level and (c) archaeal genus level.

including *Geobacter* [15,18,55]. In fact, our recent study also demonstrated hydrochars resulted in DIET mediated through surface oxygen-containing functional groups in AD of model substrate glucose [21]. It should also be noted different hydrochars had different ability to enhance methane production and methane production rate, which were consistent with the formation of different microbial communities. Further study is necessary to elucidate how different microbial communities were formed with different hydrochars. Since different hydrochars have different characters, the crucial characters that might be related with enhancement of methane production will be identified. Then hydrochar will be modify to decrease that character to see how it effects microbial communities.

Furthermore, the present study also showed hydrochars were better than GAC in enhancing methane production due to low adsorption capacity of degradable organics in HTLWW and enrichment of different microbes. Previous studies showed that GAC mainly adsorb inhibitory and recalcitrant organics in HTLWW along with some amount of degradable organics to enhance methane production [8,11,13,31]. However, the present study showed adsorption is not necessarily correlated with the enhancement of methane production and the formation of specific microbial community is more important. The application of hydrochars in AD of HTLWW is promising since hydrochar is a by-product during HTL process, and the utilization of hydrochars in AD of HTLWW would make HTL process more sustainable. In addition, hydrochars could also be tested to apply in AD of various organic wastes and wastewater.

4. Conclusions

The current research showed hydrochars increased the methane yields and methane production rates by 32–52 % and 51–84%, respectively, and adsorption was not necessarily correlated with the

enhancement of AD. Furthermore, it was noticed that hydrochars enhanced the degradation of fluorescence components, amide I and amide II, nitrogenous, aromatic and phenolic compounds as showed by 3D-EEM, FTIR and GC–MS analysis. GC–MS analysis further revealed that hydrochars reduced the number of organic species which were produced due to partly degradation of organics. Moreover, hydrochars had significant effect on microbial communities and enriched more bacteria especially syntrophic bacteria (e.g. *Syntrophomonas* and *Syntrophorhabdus*), that have capability to degrade diversified organics including aromatic and phenolic organics. The enhanced AD of HTLWW by hydrochars could be associated with DIET considering the enrichment of known bacteria involved in DIET including *Geobacter*, *Methanosaeta* and *Methanobacterium*.

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

Supplementary data to this article can be found online at <https://>

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