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Anaerobic conversion of the hydrothermal liquefaction aqueous phase: fate of organics and intensification with granule activated carbon/ozone pretreatment†

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Hydrothermal liquefaction (HTL) is considered to be a promising route for biofuel production from wet biomass. Transportation fuels and chemicals can be accessed through this process. However, a high strength aqueous phase is also produced during HTL. The conversion of the aqueous phase could significantly contribute to energy and nutrient recovery. Anaerobic fermentation has recently been proposed to biologically convert the HTL aqueous phase into biomethane. However, only a limited amount of information about the fate of the HTL aqueous phase and its conversion during anaerobic conversion is available, and it suffers from a few setbacks, including a low degradation efficiency of organics (33–64% COD) and high dilution rates (5–1000). Herein, the fates and conversion of organics in the HTL aqueous phase during anaerobic conversion were investigated by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS), gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC). MALDI-TOF-MS analysis combined with organics/nitrogen balance indicated that the remaining organics (over 30%) after anaerobic conversion might come from nitrogen-containing polymers (over 1000 Da). These nitrogen-containing polymers tended to be converted into oligomers and led to a decrease in the molecular weight (100–300 Da) by anaerobic microbes but could not be further converted into methane. Organic acids and potential inhibitors (N-heterocycles and aromatic compounds) could be completely converted into methane, but the acetogenesis of anaerobic fermentation was inhibited with an increased fermentation concentration. Ozone pretreatment and granule activated carbon (GAC) addition were integrated to improve the anaerobic conversion efficiency of the HTL aqueous phase. Ozone pretreatment significantly improved the conversion of organics and methane production by 109% through converting the inhibitors. At the same time, ozone pretreatment could convert nitrogen-containing polymers into low molecular weight organics, but organics with a molecular weight ranging from 500 to 1000 Da were produced and could not be converted by microbes or adsorbed by GAC. In comparison, GAC addition increased the methane yield more remarkably by 298% at a 2× dilution rate of the HTL aqueous phase through adsorbing inhibitors and enriching robust microbes, such as detoxification bacteria and syntrophic acetogens. Moreover, GAC adsorbed the recalcitrant organics and resulted in a removal of organics of 93.3–96.8%. Therefore, clean water with polished nutrients could be achieved through intensified anaerobic conversion, which could enhance the following biomass production and nutrient recovery through algae cultivation.

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Introduction

Hydrothermal liquefaction (HTL) is a promising route to convert wet biomass into biofuels since it sidesteps the cost-intensive dewatering and drying process.[1] Various types of biomass, including livestock manure, algae, and sludge, have been investigated to produced biocrude oil *via* HTL.[2,3] Upgrading biocrude oil has demonstrated the potential to produce liquid fuel *via* this process and obtain valuable chemicals such as ketones and oxygenated aromatics.[4,5] However, during the HTL process, 20–40% of organic compounds and 60–80% of nutrients in the feedstock are transformed into the aqueous phase.[6–9] Hence, the valorization of the aqueous phase is a critical step for maximizing the energy efficiency and nutrient recovery of the HTL process.[9]

Anaerobic conversion of the HTL aqueous phase has been recently studied[10–13] due to its higher tolerance for the HTL aqueous phase and cost-effective conversion[14] compared to other biological approaches, such as algae cultivation.[12] The HTL aqueous phase consisted of complex composition, including easily biodegradable organics (sugars and volatile fatty acids (VFAs)), potential toxic organics (furans, phenols and N-heterocyclic compounds) and high molecular weight organics.[15–17] In addition, only 40–70% of the total organic carbon in the HTL aqueous phase is quantitatively measurable based on current mass spectrometry and chromatography methods.[18] The conversion of organics in the HTL aqueous phase, including organic acids and potential inhibitors, was reported during anaerobic conversion.[10,11,19] However, 33–64% of organics in the HTL aqueous phase remained in the anaerobic slurry,[10,12,20,21] and the characteristics and conversion of these compounds have yet to be unlocked. Hence, the investigation of the fate of organics during the anaerobic conversion of the HTL aqueous phase is necessary for comprehensively understanding the energy and nutrient recovery along with the potential environmental risks of the HTL process.

Various methods have been used to improve the anaerobic conversion of the HTL aqueous phase.[10–13] Using powdered activated carbon (PAC) to adsorb potential inhibitors not only improved the methane production but also reduced the lag phase.[12] Although the equilibrium of adsorption could be achieved faster using PAC over granular activated carbon (GAC),[12] GAC can be retained in a reactor, which plays a role as a microbial carrier and does not present the risk of being lost to the effluent and/or sludge removal like PAC.[22,23] Zheng *et al.* compared the effect of zeolite, polyurethane matrices and GAC on the anaerobic conversion of the HTL aqueous phase, and GAC addition achieved the greatest methane improvement.[10] Ozone pretreatment is also a potential method to improve the anaerobic conversion of the HTL aqueous phase. The oxidative properties of ozone can increase the biodegradability of organic chemicals.[24] Upon reaction with ozone, aromatic and N-heterocyclic compounds were degraded into more biodegradable organics, such as acids and amidic compounds.[25,26] Our previous work showed that the biodegradability could be increased by 32.4% through incorporating ozone to oxidize the phenols in the HTL aqueous phase.[27] To the best of the authors' knowledge, the literature using the above measures all focused on methane production; however, an in-depth investigation of the conversion of organics of the HTL aqueous phase during anaerobic conversion has not yet been reported.

Considering all these, the present work was dedicated to revealing the fate of the main compounds in the anaerobic conversion of the HTL aqueous phase, including high molecular weight organics, potential biological inhibitors and VFAs, using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS), gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC), respectively. Furthermore, GAC addition and ozone pretreatment were applied to enhance the conversion of organics during the anaerobic conversion of the HTL aqueous phase. In particular, the effect of GAC addition on the microbial structure and functions was characterized by Illumina MiSeq sequencing. Based on the above descriptions, the conversion pathways of the HTL aqueous phase were proposed and compared with ozone pretreatment and GAC addition. Finally, we evaluated the role of anaerobic conversion on both the HTL energy recovery and nutrient recycling.

Experimental section

HTL of swine manure

HTL was conducted using a pilot-scale continuous HTL reactor (Snapshot Energy Inc.). The feedstock for HTL reactions was swine manure which was obtained from a grower-finisher barn. The organic composition, including the crude fat, crude protein, carbohydrate, hemicellulose, cellulose and lignin content of swine manure, was analyzed based on AOAC standard methods. The ash content was measured through burning the swine manure in a muffle furnace at 600 °C. HTL was conducted at 270 ± 10 °C with a retention time of 1 h and a total solid content of 13%. The HTL aqueous phase was collected and filtered using 0.45 μm filters before being used.

Anaerobic conversion of the HTL aqueous phase

The inoculum for anaerobic conversion was obtained from an anaerobic digester in the Urbana & Champaign Sanitary District (Urbana, Illinois, USA). A previously reported synthetic wastewater (1 g COD per L) was used to cultivate the inoculum.[28] The cultivation of the inoculum stopped when methane production completed. Then the inoculum was washed using DI water and centrifuged (3000 rpm, 10 min).

Anaerobic batch treatment was conducted using 160 mL serum bottles in triplicate. The experiment was conducted at a temperature of 37 °C with a water bath. At the beginning of anaerobic conversion, 20 mL sludge was added to the bottles, and 0.5 g $NaHCO_3$ per g COD was added as a buffer. As shown in Table 1, the effect of ozone pretreatment (OP), GAC addition (GAC) and a combination of OP and GAC (OG) on the anaerobic conversion of the HTL aqueous phase was studied and

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Table 1 Experiments set for the anaerobic conversion of the HTL aqueous phase

Experimental set	HTL aqueous phase (g COD per L)	Inoculum	Ozone pretreatment	GAC addition
OP_1 [a]	5	●	●	○
OG_1 [b]	5	●	●	●
GAC_1 [c]	5	●	○	●
C_1 [d]	5	●	○	○
OP_2	10	●	●	○
OG_2	10	●	●	●
GAC_2	10	●	○	●
C_2	10	●	○	○
OP_r [e]	10	●	●	○
OG_r [e]	10	●	●	●
GAC_r [e]	10	●	○	●
C_r [e]	10	●	○	○
OP_3	20	●	●	○
OG_3	20	●	●	●
GAC_3	20	●	○	●
C_3	20	●	○	○

[a] OP = ozone pretreatment. [b] GAC = GAC addition. [c] OG = combination of ozone pretreatment and GAC addition. [d] C = control group. [e] Repeat test was another independent batch test of the anaerobic conversion of the HTL aqueous phase for long-term operation. ● = With inoculum/ozone pretreatment/GAC addition; ○ = without inoculum/ozone pretreatment/GAC addition.

compared to the results from the control (C). The batch test was conducted at three different fermentation concentrations, including 5 g COD per L (OP_1, OG_1, GAC_1, and C_1), 10 g COD per L (OP_2, OG_2, GAC_2, and C_2), and 20 g COD per L (OP_3, OG_3, GAC_3, and C_3). OP was performed using a portable ozone producer (SATA 03601), in which 2.32 mg min^{-1} ozone was produced. An ozone dosage of 2.1 mg O_3 per mL HTL aqueous phase was applied based on our previous research.[27] The GAC (Calgon F-400) addition was 20 g L^{-1} which could adsorb about 0.25 g COD in the HTL aqueous phase per g GAC. Anaerobic conversion experiments were conducted at 37 °C in a shaking incubation chamber. The gas volume was measured daily using a glass syringe, and the gas content was measured by using a gas chromatograph.

To investigate the effects of GAC and ozone pretreatment on the anaerobic conversion of the HTL aqueous phase for long-term operation, another independent batch test was conducted (Table 1). OP_r, OG_r, GAC_r, and C_r used the same parameters as those of OP_2, OG_2, GAC_2 and C_2, respectively. A mixture of the effluent at 10 g COD per L was centrifuged at 3000 rpm for 10 min, and the sludge and GAC were collected. The sludge was then washed using 30 mL DI water and then subsequently centrifuged. The centrifugation and washing were repeated 3 times. The collected sludge was used as the inoculum for the repeated test, which was also conducted at a concentration of 10 g COD per L.

The kinetic analysis of anaerobic conversion was investigated using the modified Gompertz model (1)[11]

$$P = P_s \exp[-\exp(R_m \times e/P_s \times (\lambda - t) + 1)] \quad (1)$$

where P is the accumulative methane volume (mL g^{-1} COD); P_s is the maximum accumulative methane volume (mL g^{-1} COD); R_m is the maximum methane production rate (mL per g COD per d); e is exp (1) = 2.71828; λ is the lag phase (d); and t is the reaction time (d).

Organics and nitrogen balance calculation

In order to calculate the nitrogen balance of the anaerobic conversion of the HTL aqueous phase, the following assumptions were made. Nitrogen in the HTL aqueous phase was assumed to consist of organic nitrogen and ammonia because of the limited nitrite nitrogen (3.5–0.1%).[29,30] During anaerobic conversion, no nitrogen was lost in the form of gas, and degraded organic nitrogen was converted into ammonia nitrogen. Ammonia adsorbed by GAC (TA_{ad}, g L^{-1}) achieved by the control group which was conducted without an inoculum (20 g per LGAC addition, 10 g COD per L, 37 °C) (Fig. 1S†); Percentage of degraded organic nitrogen (PN_d,%) = (ammonia in effluent (TA_{out}, g L^{-1}) − initial ammonia (TA_{in}, g L^{-1}) + adsorbed ammonia (TA_{ad}, g L^{-1}))/initial total nitrogen (TN_{in}, g L^{-1}) × 100%; Percentage of undegraded organic nitrogen (PN_{ud}, %) = (effluent total nitrogen (TN_{out}, g L^{-1}) − effluent ammonia (TA_{out}, g L^{-1}))/initial total nitrogen (TN_{in}, g L^{-1}) × 100%; Percentage of adsorbed organic nitrogen and growth of microbes ($PN_{ad\&m}$, %) = 100% − initial ammonia (PA_{in}, %) − degraded organic nitrogen (PN_d,%) − undegraded organic nitrogen (PN_{ud}, %).

The organic balance was calculated based on the COD values and the following assumptions. The organics produced *via* methane production were calculated based on a theoretical methane yield of 350 mL g^{-1} COD.[31] The growth of microbes was calculated *via* an acetate methane production pathway (2)[32] because acetate was the primary organic compound in the HTL aqueous phase (over 20% COD).

$$CH_3COO^- + 0.018NH_4^+ + 0.027CO_2 + 0.928H_2O = 0.018C_5H_7O_2N + 0.955CH_4 + 0.982HCO_3^- \quad (2)$$

Percentage of organics for methane production and microbes ($PO_{m\&m}$, %) = methane yield (Y_m, mL L^{-1})/theoretical methane yield (mL g^{-1} COD)/ratio of organics for methane production to organics for the sum of growth of microbes and methane production (f, g COD per g COD)/(initial COD (COD_{in}, g L^{-1}) × 100; Percentage of organics oxidized by ozone (PO_{oz}, %) = (initial COD (COD_{in}, g L^{-1}) − COD after ozone treated (COD_{oz}, g L^{-1}))/initial COD (COD_{in}, g L^{-1}) × 100%; Percentage of residual organics (PO_r) = COD in effluent (COD_{out})/initial COD (COD_{in}, g L^{-1}) × 100%; Percentage of adsorbed (PO_{ad}, %) = 100% − percentage of organics oxidized by ozone (PO_{oz}, %) − percentage of residual organics (PO_r) − percentage of organics for methane production and growth of microbes ($PO_{m\&m}$, %).

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Analytical methods for organics in the HTL aqueous phase and the microbial structure

Quantification of acids in the liquid samples was conducted using a high-performance liquid chromatograph (HPLC) (Shimadzu, Japan). The HPLC was equipped with a refractive index detector and an Aminex HPX-87H column (Bio-Rad, USA). The organic compounds in the HTL aqueous phase were analyzed using a GC-MS system (7890A, Agilent Technologies, USA). The procedure for the GC-MS analysis and data interpretation was described in a previous study.[10] Water quality analysis, including COD, ammonia and total nitrogen content (TN), was conducted according to standard methods.[33] The high molecular weight organics of the raw HTL aqueous phase and the effluent from anaerobic conversion were measured by MALDI-TOF-MS. MALDI-TOF-MS was conducted using a Brucker Auto Flex speed MALDI system (Bremen, Germany). Ultra-stable 2 kHz electronics for a TOF analyzer, a detector and an ion source fully enabled a 1–2000 Hz data acquisition rate in MS mode. A scan was conducted ranging from 100 to 100 kDa on the HTL aqueous phase. DCTB was used as the matrix for the samples. Calibration of the instrument was carried out before the measurement with Bruker peptide mixtures.

Microbial samples from the inoculum and repeated GAC_r tests were collected to evaluate the microbial structure. DNA extraction from the samples was carried out using a FastDNA™ SPIN Kit for Soil (MP Biomedicals, LLC) according to the manufacturer's instructions. Sequencing of the extracted DNA was carried out by the University of Illinois Keck Center using Illumina MiSeq sequencing combined with Fluidigm sample preparation. Primer pair V4-515 F–V4-806 R was used to amplify the V4 region of the 16 S rRNA gene of the bacteria, and the primer pair ArchaeaF349–ArchaeaR806 was used to amplify the 16 S rRNA gene of the archaea. The raw reads were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database. Raw fastq files were demultiplexed and quality-filtered using Quantitative Insights Into Microbial Ecology (QIIME). The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed using a RDP Classifier (http://rdp.cme.msu.edu/) against the SILVA (SSU115) 16S rRNA database at a confidence threshold of 70%.

Results and discussion

Characterization of the HTL feedstock and the HTL aqueous phase

The characterization of swine manure is shown in Table 2. Algae[34] and cornstalk,[35] which are two typical HTL feedstocks, were selected for comparison to elucidate the effect of the feedstock composition on the HTL aqueous phase. The crude protein content of swine manure was higher than that of cornstalk but lower than that of algae. Cornstalk had the highest carbohydrate content and mainly consisted of lignocellulose. Crude fat in swine manure was 18.6% which was higher than that in both algae and cornstalk. From the composition analysis, swine manure is a suitable feedstock for biocrude oil production through HTL. The potential for converted biomass components to be converted into biocrude oil through HTL followed the trend, lipids > proteins > carbohydrates.[35]

Table 2 Chemical compositions of biomass for HTL

Chemical composition[a]	Swine manure	Algae[34]	Cornstalk[35]
Ash (%)	13.0 ± 2.3	6.3 ± 0.04	4.0
Crude fat (%)	18.6 ± 0.3	14.1 ± 0.3	0.7
Crude protein (%)	25.3 ± 2.3	52.4 ± 1.2	10.8
Carbohydrate[b] (%)	43.2 ± 0.4	27.2 ± 0.4	84.5
Hemicellulose (%)	25.9 ± 2.9	Na[c]	29.7
Cellulose (%)	8.7 ± 2.3	Na	45.1
Lignin (%)	2.7 ± 3.7	Na	5.7

[a] Dry weight basis. [b] Calculated by deference, carbohydrate (%) = 100 − crude fat (%) − crude protein (%) − ash content (%). [c] Na = Not applicable.

The characteristics of the HTL aqueous phase are also tightly related to the selection of the feedstock. Table 3 summarizes the chemical composition of the HTL aqueous phase generated from swine manure, algae[11] and cornstalk,[19] respectively. HTL reactions were performed at similar temperatures (260–280 °C). However, the HTL aqueous phase composition showed significant differences which was related to the selection of the HTL feedstock. Compared with cornstalk, a higher total nitrogen and ammonia concentration in the HTL aqueous phase for swine manure and algae was observed, which was attributed to the high protein content in the feedstock. This suggested that nutrient recycling by algae cultivation could be a feasible pathway for the valorization of the HTL aqueous phase generated from swine manure and algae. However, an abundance of N-heterocyclic compounds was observed in the HTL aqueous phase in swine manure and algae, which could reach up to 18.77% and 31.60%, respectively. Pham *et al.* reported the toxicity of these nitrogenous organic compounds and a low conversion rate through algae cultivation, which could only remove 30% of these inhibitory compounds.[36] In addition, aromatic compounds (benzoic acid derivatives and phenols) were also observed in the HTL aqueous phase, which could significantly inhibit algal growth even at low concentrations.[37] From this aspect, anaerobic conversion could be a critical step, in which it converts organics into bioenergy fuel and enhances nutrient recycling by acting as a detoxification agent for algae cultivation.

Biomethane production of the HTL aqueous phase

Fig. 1 shows the methane production of the HTL aqueous phases generated from swine manure with concentrations of 5, 10 and 20 g COD per L, respectively. The methane yield at a concentration of 5 g COD per L demonstrated a similar performance in all treatments (Fig. 1a), which ranged from 213–219 mL g^{-1} COD, and no obvious inhibition was observed. A significant decrease (by 47.9%) of the methane yield was observed in the control group (Fig. 1b) when the HTL aqueous phase concentration increased to 10 g COD per L. The GAC_2

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Table 3 Chemical compositions of the HTL aqueous phase generated from biomass

Feedstock HTL conditions[b]	Swine manure[a] 270 °C, 13%	Algae[11] 260 °C, 25%	Cornstalk[19] 260 °C, 20%
Total nitrogen (g L^{-1})	1.85 ± 0.1	80	1.0 ± 0.1
Ammonia nitrogen (g L^{-1})	Na	18.8	Na[c]
COD (g L^{-1})	39.8 ± 0.1	52.0	76.2 ± 1.6
Formic acid (mg L^{-1})	Na	Na	8509 ± 1542
Lactic acid (mg L^{-1})	2160 ± 24	Na	9758 ± 1392
Acetic acid (mg L^{-1})	7612 ± 48	Na	22 336 ± 2476
Propionic acid (mg L^{-1})	2472 ± 80	Na	2730 ± 856
Butyric acid (mg L^{-1})	2304 ± 28	Na	9072 ± 2136
Valeric acid (mg L^{-1})	380 ± 2	Na	Na
Major components[d] (%)			
Short chain organic acid (2 < C < 6)	50.34%	36.81%	Na
Long chain acids (C > 12)	5.49%	Na	Na
N-Heterocyclic compounds	18.77%	31.60%	1.80%
Alcohols	4.31%	Na	Na
Benzoic acid derivatives	4.75%	2.03%	Na
Phenols	2.13%	0.21%	25.10%
Straight amide derivatives	2.70%	6.31%	Na
Amino acid	6.78%	7.07%	Na
Ketones	Na	11.60%	2.18%
Furfurals	Na	Na	29.64%
Oxygenates (cyclic and straight)	Na	6.70%	8.64%
Total	95.29%	92.29%	67.36%

[a] $a \pm b$ represents the mean and standard deviation calculated from $n \geq 2$. [b] Represents the HTL temperature X °C and total solid content Y%. [c] Na = Not applicable. [d] Relative area (%) identified by GC-MS.

and OG_2 groups maintained a stable methane yield whereas the OP_2 group decreased by 11.1% compared with OP_1. A further increase in the concentration of the HTL aqueous phase to 20 g COD per L (Fig. 1c) led to an anaerobic conversion lasting over 100 days. Serious inhibition was found in the C_3 trial, in which the methane yield decreased by 83.6% compared with C_1. With the inclusion of ozone pretreatment, the methane yield of the OP_3 group significantly improved by 105.6% compared with C_3. This should be related to the oxidation of the inhibitors during ozone pretreatment. Although only part of the N-heterocyclic compounds in the HTL aqueous phase were removed by ozone,[27] the complete conversion of phenols enhanced the anaerobic fermentation by avoiding the interaction inhibition of N-heterocycles and phenols.[38] The methane yield of the GAC_3 and OG_3 groups was 212 ± 7 and 202 ± 3 mL g^{-1} COD, respectively, which was 298.1% and 279.2% higher compared with C_3. This indicated that the GAC significantly remediated the inhibition of the HTL aqueous phase, and the improvement was attributed to the adsorption of toxic compounds in the HTL aqueous phase. The repeated test indicated the long-term performance of anaerobic conversion of the HTL aqueous phase with the incorporation of enriched sludge and GAC (Fig. 1b). The OG_r and GAC_r groups had a methane yield of 208 and 217 mL g^{-1} COD, respectively. These methane yields remained the same as the first batch (OG_2 and GAC_2).

The kinetic analysis based on the modified Gompertz model indicated that the increased HTL aqueous phase addition led to a significant inhibition (Table 1S†). Specifically, with the increase of the concentration of the HTL aqueous phase from 5 g COD per L to 10 g COD per L and 20 g COD per L, the lag-phase of the control group increased from 5.9 d to 12.2 d and 36.3 d, respectively. An interesting result was the slight increase of the lag-phase in OP and OG compared with the control group. Compared with the control group, the addition of GAC significantly decreased the lag-phase to 9.5 and 19.3 d at 10 and 20 g COD per L per d, respectively. This result can be attributed to the adsorption ability of GAC which decreased the concentration of the inhibitors. A decrease of the maximum methane production rate with an increase of the wastewater concentration was observed in the control group, which was 15.7, 7.3 and 1.1 mL per d per g COD, respectively. On the other hand, a significant improvement of the methane production rate was observed in the ozone pretreatment group. The methane production rate was improved by 14.0–118.2% with an increased concentration of the HTL aqueous phase. Similar results were also observed in the GAC group, in which an increased methane production rate within the range of 10.2–423.4% was observed. The highest improvement of the methane production rate (increased by 472.7%) was observed in the combination of GAC addition and the ozone pretreatment group. The kinetic analysis showed that the lag-phase was significantly reduced by 45.9% and 61.1% in the OG_r and GAC_r groups, respectively (Table 1S†). This suggested that the addition of GAC accelerated the adaptation process of microbes on the degradation of the HTL aqueous phase. On the other hand, without the presence of GAC, the methane yield decreased by 24.4% and 36.7% in the OP_r and C_r groups, respectively. Moreover, a significant decrease in the methane production rate (27.9–39.7%) and a slight increase in the lag-phase (4.5–5.7%) were also observed.

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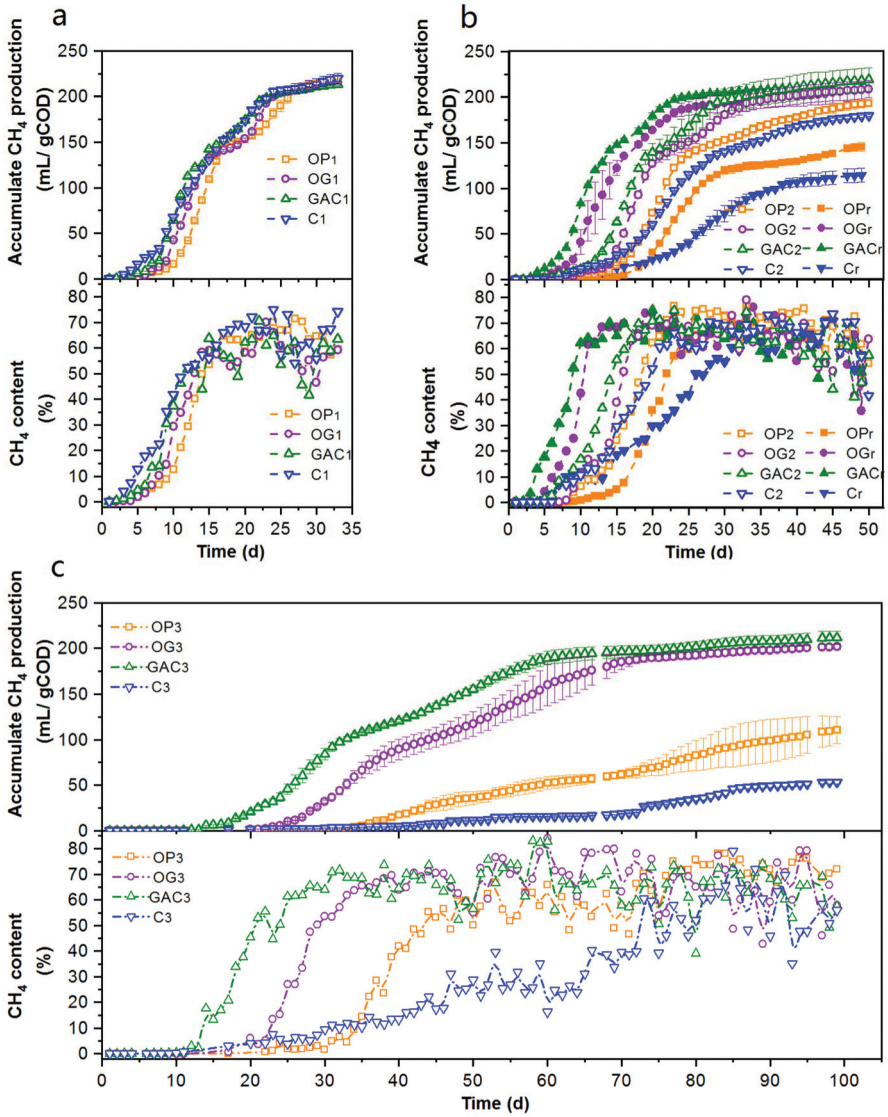

Fig. 1 Methane production with ozone pretreatment (OP), combination of ozone pretreatment and GAC addition (OG), GAC addition and control (C) group at 5 g COD per L (a), 10 g COD per L (b), repeated test 10 g COD per L (b) and 20 g COD per L (c).

Methane production using different methods to improve the efficiency of the anaerobic conversion of HTL aqueous phases was summarized (Table 4), including the addition of GAC, PAC, polyurethane matrices, and zeolite, struvite recovery, and extraction with an organic solvent.[10,12,20,21,39] Ozone treatment indicated its promising potential as both the methane yield and the methane production rate improved; however, the compatibility between ozone and anaerobic conversion and its economic feasibility needs to be further evaluated. The GAC addition in this study led to the highest improvement of the methane yield (298%) at 2 dilution rates, compared with the literature.

Anaerobic conversion of organic acids, inhibitors and high molecular weight organics in the HTL aqueous phase

With the increase of the HTL aqueous phase concentration from 5 g COD per L to 20 g COD per L, the conversion of organics of the control group decreased from 69.8% to 27.4% (Table 5). This indicated an inhibition of anaerobic conversion, which is also supported by the reduced conversion efficiency of acids. Specifically, propionic acid, butyric acid and valeric acid accumulated in C_3. Similar results were also observed in the anaerobic conversion of the algae HTL aqueous phase, in which acetogenesis was the rate-limiting step.[11] Compared with the control group, a higher conversion efficiency of COD and organic acids was achieved in the ozone pretreatment group at 10 g COD per L and 20 g COD per L HTL aqueous phase. This suggested that ozone pretreatment enhanced the acid conversion of the HTL aqueous phase. As for the groups including GAC addition, most of the acids were converted into methane. This indicated that the addition of GAC favoured acetogenesis and methanogenesis. An interesting result was that much higher organic conversion rates were observed in the OG_1 (92.3 ± 0.1%) and GAC_1 (96.8 ± 0.3%)

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Table 4 Performance of anaerobic conversion of the HTL aqueous phase in the literature and this study

Feedstock	HTL condition[a]	Anaerobic conversion[b]	Measures	Methane yield (mL g^{-1} COD)	COD removal%	Conclusion	Ref.
Spirulina	300 °C, 0.5 h, 20%	×15, 5.9 g L^{-1}	GAC 2 g L^{-1}	245	52	Improve methane yield by 13%	10
Spirulina	300 °C, 0.5 h, 20%	×15, 5.9 g L^{-1}	PM 20 g L^{-1}	278	43	Improve methane yield by 29%	10
Spirulina	300 °C, 0.5 h, 20%	×15, 5.9 g L^{-1}	Zeolite 2 g L^{-1}	227	37	Improve methane yield by 5%	10
Nannochloropsis	320 °C, 0.5 h, 20%	×97, 1 g L^{-1}	Struvite recovered	182	59	Improve methane yield by 250%	20
Wastewater algae	260–320 °C, 1 h, 25%	×∼1000, ∼0.04 g L^{-1}	—	Na	44–61	300 °C leads to the highest lag phase and the smallest production rate	11
Swine manure	Na	×7.5–30.3, 3.4–13.8 g L^{-1}	PAC	∼150–175	45–55	Shortening the lag phase and allowing AD to occur at high concentration	12
Rice straw	170–320 °C, 0.5–4 h, 10%	×15–39, 0.75 g L^{-1}	—	217–314	Na	More recalcitrant organics were produced with the increase of HTL temperature	13
Rice straw	280, 0.5 h, 6%	×28, 0.75 g L^{-1}	PE extraction	235	Na	Improve methane yield by 28%	39
Corn straw	260 °C, 0 h, 20%	× 5, 8 g L^{-1}	—	142	65	Stable methane production rate could be obtained in anaerobic high-rate reactors	52
Swine manure	270 °C, 1 h, 13%	×2, 20 g L^{-1}	GAC 20 g L^{-1}	212	93	Improve methane yield by 298%, enhance the acid conversion and reduce the lag-phase	This study
Swine manure	270 °C, 1 h, 13%	×2, 20 g L^{-1}	Ozone 2.1 g L^{-1}	111	44	Improve methane yield by 109% and methane production rate	This study

[a] Temperature, retention time, and total solid. [b] Dilution rate and initial concentration. PM, polyurethane matrices; PE: petroleum ether.

Table 5 Biochemical conversion of organics in different groups

Conversion efficiency[a] (%)	HTL aqueous phase 5 g COD per L				HTL aqueous phase 10 g COD per L				HTL aqueous phase 10 g COD per L				HTL aqueous phase 20 g COD per L			
	OP_1	OG_1	GAC_1	C_1	OP_2	OG_2	GAC_2	C_2	OP_r	OG_r	GAC_r	C_r	OP_3	OG_3	GAC_3	C_3
COD	69.0 ± 0.6	92.3 ± 0.1	96.8 ± 0.3	69.8 ± 1.1	67.8 ± 2.4	92.2 ± 0.9	96.2 ± 0.8	60.6 ± 3.1	62.2 ± 2.5	91.6 ± 0.6	94.0 ± 1.6	38.7 ± 1.8	44.5 ± 2.9	91.9 ± 1.1	93.3 ± 0.2	27.4 ± 6.2
Formic acid	100	100	Na[b]	Na	100	100	Na	Na	100	100	Na	Na	100	100	Na	Na
Acetic acid	88.1 ± 6.2	86.3 ± 9.1	86.6 ± 2.4	87.9 ± 3.9	89.4 ± 4.0	95.5 ± 1.3	95.2 ± 3.5	68.7 ± 12.0	82.1 ± 5.7	96.5 ± 0.6	100	62.4 ± 8.2	38.6 ± 22.7	97.5 ± 0.	97.5 ± 0.5	35 ± 3.6
Lactic acid	77.6 ± 1.4	75.4 ± 1.2	77.2 ± 4.9	84.1 ± 0.4	88.1 ± 1.8	89.3 ± 0.3	95.8 ± 5.9	89.6 ± 2.3	96.8 ± 4.5	100	100	100	100	93.5 ± 1.6	98.9 ± 1.5	91.6 ± 2.5
Propionic acid	100	100	100	100	100	100	100	100	11.8 ± 23.2	100	100	−2.8 ± 3.4	16.4 ± 37.1	100	100	−20.2 ± 3.2
i-Butyric acid	100	100	100	100	43.9 ± 22.7	100	100	52.4 ± 3.2	100	100	100	−16.7 ± 7.8	100	100	100	−156.5 ± 34.2
n-Butyric acid	100	100	100	100	100	100	100	100	95.7 ± 3.1	100	100	97.1 ± 0.1	94.1 ± 1.4	95.4 ± 4.3	97.1 ± 0.3	6.5 ± 86.3
Valeric acid	100	100	100	100	100	100	100	100	100	100	100	100	79.9 ± 4.9	100	100	−36.1 ± 13.4

[a] Minus conversion efficiency indicated accumulation. [b] Na = not applicable.

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groups at 5 g COD per L, but no significant difference in the methane yield in all groups was observed. This can be explained by the fact that some organic compounds were not converted into methane but adsorbed by GAC. As for the repeated test, OG_r and GAC_r showed a conversion efficiency of 91.6% and 94.0%, respectively, and they were slightly lower than the first batch test. The lower removal of organics may be due to the decreased adsorption ability of GAC. The higher conversion efficiency of acetic acid was found in the GAC group, which indicated a strengthened methanogenesis reaction. This may be due to the formation of biofilms on the GAC and better enriched microbes. In contrast, the OP_r and C_r groups showed a decrease in the conversion of organics resulting from the accumulation of propionic acid and acetic acid.

The repeated test (10 g COD per L) was investigated as a representative to reveal the fates of organics during anaerobic conversion because of the significant difference. The conversion of alcohols, long-chain acids, and N-heterocyclic and aromatic compounds was revealed by the peak area changes based on GC-MS analysis (Fig. 2), which contained information regarding the main inhibitors to anaerobic conversion present within the HTL aqueous phase. Most of the potential inhibitors were converted by over 80%. A similar result was also observed in the anaerobic conversion of the HTL aqueous phase from cornstalk.[19] A higher conversion efficiency of aromatic and organic nitrogen-containing compounds was observed in the ozone pretreated groups compared with the control group, which proved that the ozone pretreatment enhanced the conversion of inhibitors. This result was significant because most of these compounds have been classified as being highly cytotoxic,[36] and their mineralization makes the effluent of anaerobic conversion of the HTL aqueous phase an appropriate nutrient source for algae cultivation.[40] The addition of GAC indicated that a higher conversion efficiency of long-chain organic acids (C > 12) and N-heterocyclic and aromatic compounds was achieved when compared with the control group. In particular, compared with the limited conversion efficiency (75.5%) of 3-(3-hydroxyphenyl)propionic acid in the control group, complete conversion of this compound was observed upon GAC addition.

One problem that needs to be addressed is understanding the composition of the remaining recalcitrant organics after anaerobic conversion. As shown in Table 5 and Fig. 2, over 80% of the organic acids and potential inhibitors were converted during anaerobic conversion. However, this result did not correspond to the limited conversion of organics observed in the control and ozone pretreated groups. A possible explanation was that these recalcitrant compounds were not detected by GC-MS, possibly due to their high molecular weight. This hypothesis is supported by a previous study which confirmed the presence of a considerable amount of high molecular weight organic compounds in the HTL aqueous phase.[39] The balance of organics and nitrogen during the anaerobic conversion of the repeated test was investigated to further understand the characteristic of the residual organics during the conversion process (Fig. 3). A close relationship between the adsorption of nitrogen and the conversion of organics was observed, which indicated that these residual

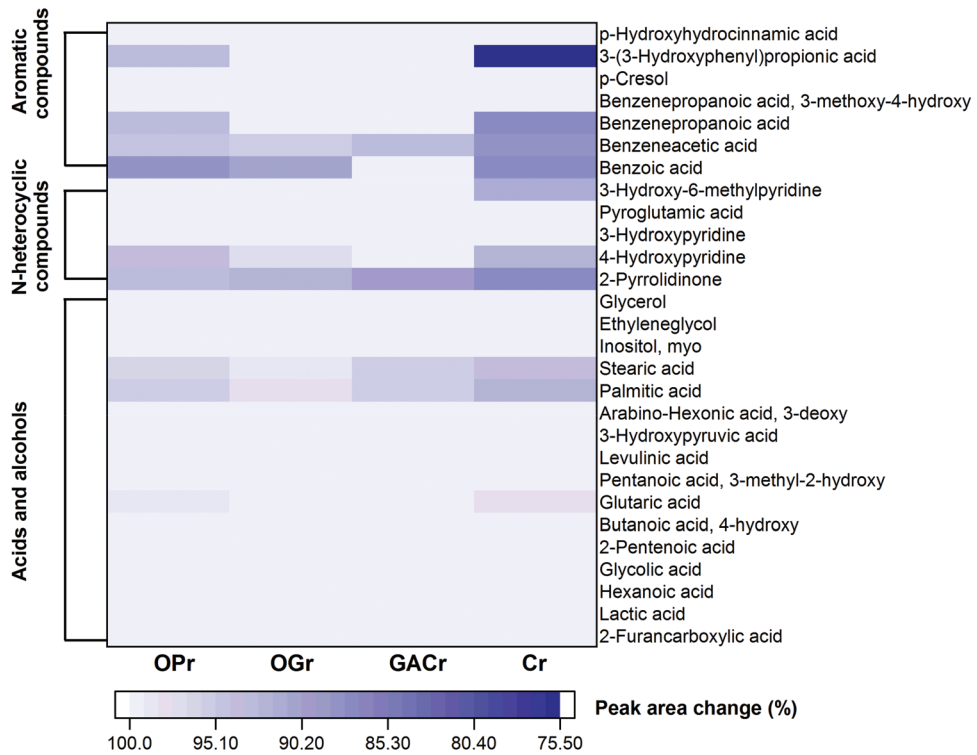

Fig. 2 Peak area changes of metabolism intermediates based on GC-MS analysis in the repeated test (10 g COD per L).

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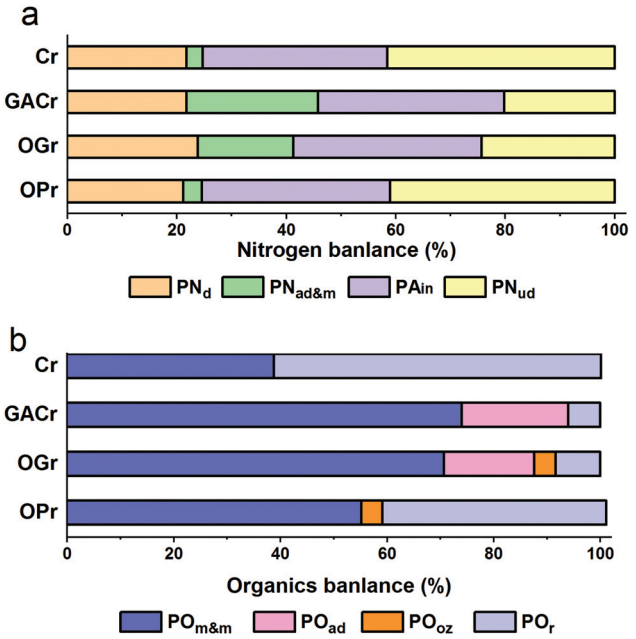

Fig. 3 Organics and nitrogen balance in the repeated anaerobic conversion test (10 g COD per L). (Percentage of degraded organic nitrogen (PN_d, %), adsorbed organic nitrogen & microbes growth ($PN_{ad\&m}$, %), initial ammonia (PA_{in}, %), undegraded organic nitrogen (PN_{ud}, %); Percentage of organics for methane and microbes growth ($PO_{m\&m}$, %), adsorbed organics (PO_{ad}, %), organics oxidized by ozone (PO_{oz}, %), organics residue (PO_r)).

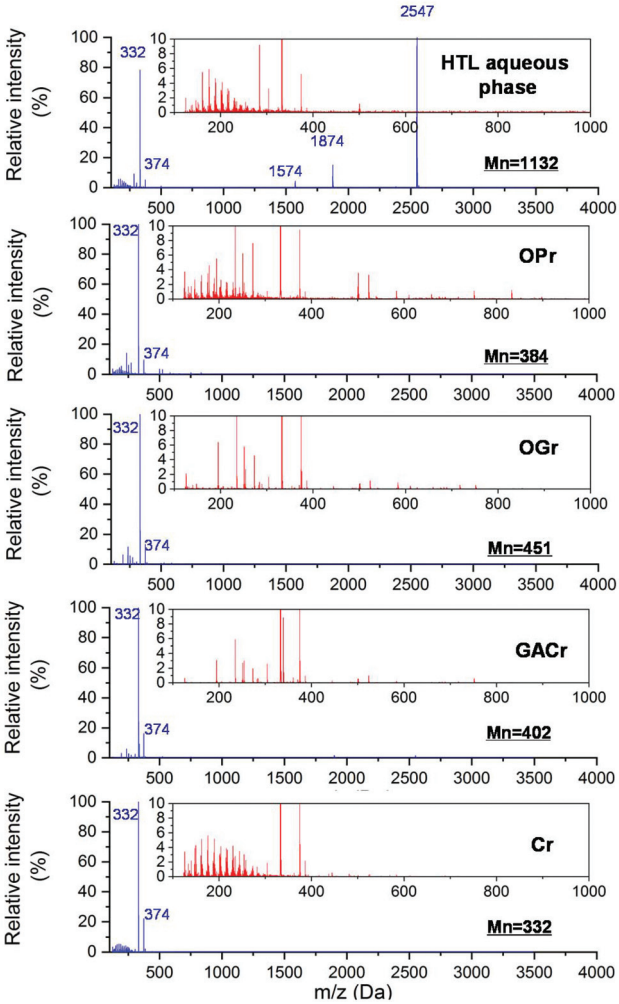

Fig. 4 MALDI-TOF-MS analysis of the HTL aqueous phase, effluent of repeated anaerobic conversion test with ozone pretreatment (OP_r), combination of ozone pretreatment and GAC addition (OG_r), GAC addition (GAC_r) and control (C_r) in the repeated test (10 g COD per L).

organics may consist of high molecular weight nitrogen organics, which were hypothesized to originate from the Maillard reactions involving carbohydrates and proteins, including oligosaccharides, polysaccharides (starch, cellulose, hemicellulose, and glycogen) and peptides.[41–44] One previous study verified the distribution of abundant high molecular weight nitrogen organics (carbon numbers reaching up to 40) in the HTL aqueous phase from algae.[15] These recalcitrant and water-soluble nitrogen organics may negatively impact the environment.

MALDI-TOF-MS was used for high molecular weight compound analysis, which is respected as a routine analytical tool for polymers, and it allows for the detection of large molecules (up to 100 kDa).[45,46] The scan of the HTL aqueous phase *via* MALDI-TOF-MS showed no signal over 4 kDa which indicated the absence of chemicals in that molecular weight range. As shown in Fig. 4, peaks at 1574, 1874 and 2547 Da were observed in the HTL aqueous phase, which confirmed the existence of potential polymers. After anaerobic conversion, a clear shift in the MW distribution was observed. These peaks disappeared after anaerobic conversion, indicating the degradation of these polymers. As suggested in M_n (number average molar mass), the degradation of these polymers resulted in a decrease of the molecular weight. The degradation process of high molecular weight compounds may be similar to that for lignin which is first degraded to smaller units by extracellular enzymes.[47] The MALDI mass spectrum of C_r and OP_r showed a noisy background in the low mass range of 100–300 Da, which indicated the presence of hydrolysis products from macromolecules (>1000 Da). These organics may consist of oligomers, which were also observed in the HTL aqueous phase generated from algae.[17] These degraded products which were derived from the macromolecules may not be further converted into methane. This was confirmed by their limited contribution to the methane production yield and limited conversion of organics in C_r and OP_r (Fig. 3).

The GAC added group, including GAC_r and OG_r, showed a much clearer background in the range of 100–300 Da, indicating the adsorption of these hydrolysis products. The higher M_n in the GAC added group confirmed this result, indicating that the lower molecular weight compounds were adsorbed. Hence, the microbes played a critical role in the process, which resulted in a decrease in the molecular weight, and the GAC further adsorbed these compounds. The decrease of the molecular weight is critical because it enhances the adsorption of

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chemical compounds to GAC.[48] The presence of the remaining organics, mainly with peaks higher than 200 Da, suggested the selective adsorption of GAC. The organics at peaks of 332 and 374 Da need further investigation. Peaks in the range from 500 to 1000 Da were found in OP_r and OG_r, and these organics were attributed to the ozone pretreatment which converted the macromolecules into low molecular weight chemicals (Fig. 2S†), which was observed in the ozone treatment of the pulp mill effluent.[49] However, these compounds were not further converted by microbes, and they were not effectively adsorbed by GAC either. This result supported the lower conversion efficiency of organics in the OGr group. From this aspect, the ozone treatment showed a negative effect on nitrogen containing polymer conversion by producing these recalcitrant products.

As discussed above, GAC addition achieved the highest conversion of organics of 93% in this study, which was much higher than the previously reported values (Table 4). This result was partly attributed to the strengthened adsorption of GAC (20 g L^{-1}) compared with the literature (2 g L^{-1}).[10] In addition, microbial enrichment by GAC also enhanced the conversion of organics, which was confirmed by a decrease of the lag-phase and an improvement of acid conversion in the repeated test (Table 1S† and Table 5). In order to reveal the effect of microbial enrichment by GAC on the conversion of inhibitors, the changes of the bacterial and archaea communities in the inoculum and GAC_r were investigated at the family level (Fig. 5). Compared with the inoculum, an increase of the relative abundance of Anaerolineaceae, Burkholderiaceae, Comamonadaceae, Gracilibacteraceae, Hyphomonadaceae, Lachnospiraceae, Lentimicrobiaceae, Limnochordaceae, Porphyromonadaceae, Peptococcaceae, Rhodobacteraceae, Rikenellaceae, and Peptococcaceae was observed. In particular, the abundance of the families Anaerolineaceae, Burkholderiaceae and Peptococcaceae in GAC_r reached 12.9%, 6.3% and 3.1%, respectively. The Anaerolineaceae family was reported to be attributed to the biodegradation of high-concentration phenolic compounds in an anaerobic sequencing batch reactor.[50] Some species from the family Burkholderiaceae can be used for the bioremediation of crude oils, herbicides, and ether derivatives used as gasoline additives.[51] The family Peptococcaceae was also observed in the anaerobic conversion of the HTL aqueous phase generated from cornstalk, which was related to benzene degradation.[19] The increased relative abundance of these bacteria indicated the enrichment of microbes related to the detoxification of the HTL aqueous phase. Another interesting change was the increase of syntrophic bacteria in GAC_r, including Synergistaceae, Syntrophaceae, Syntrophobacteraceae, Syntrophomonadaceae, Syntrophorhabdaceae and Thermoanaerobacteraceae, which mostly function as promoters of acetogenesis.[52,53] This result suggested that GAC could enhance acetogenesis by enriching syntrophic acetogens, which also corresponded to the effective acid conversion in the GAC added group. As for the archaea communities (Fig. 5b), a significant decrease of the acetoclastic methanogen Methanosaetaceae was observed, while Methanomicrobiaceae and Methanosarcinaceae, which can use hydrogen to produce methane, showed an increased abundance. This result can be explained by the higher abundance of syntrophic acetogens in

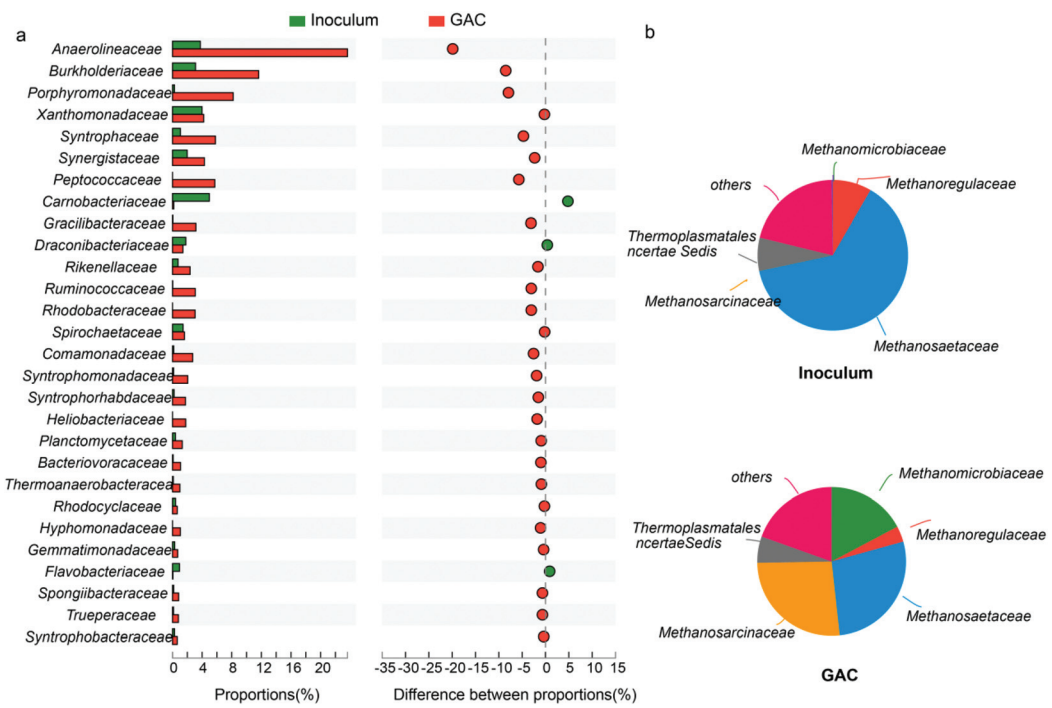

Fig. 5 Taxonomic classification of Illumina MiSeq sequencing from bacterial (a) and archaea (b) communities in the inoculum and GAC_r at the family level. (Relative abundance was defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample).

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GAC_r. In addition, the observed efficient acetate conversion in GAC resulted from the family Methanosarcinaceae, which outcompetes Methanosaeta under high concentrations of acetic acid.[54]

Outlook on waste biorefinery *via* combined HTL and intensified anaerobic conversion

Based on the concept of biorefinery, a combination of HTL, anaerobic conversion and algae cultivation was proposed (Fig. 6). The HTL mechanical pathway for biomass conversion is a multi-stage process, which involves hydrolysis, polymerization, decarboxylation, dehydration and deamination.[1,55] Four main products could be achieved after HTL. Transportation fuels (biocrude oil), hydrochar (solid phase) and carbon dioxide for algae cultivation (gas phase) could be further accessed by upgrading, respectively. Substantial organic acids and aromatic and N-heterocyclic compounds were transferred into the aqueous phase. In particular, the sugars and amino acids produced from hydrolysis react together *via* the Maillard reaction, and lead to the formation of nitrogen containing polymers.[55] Nitrogen containing polymers are also transferred into the HTL aqueous phase. Strengthened anaerobic conversion could further valorize the HTL aqueous phase. Fig. 6 shows the mechanical conversion pathway of the HTL aqueous phase with ozone pretreatment, GAC addition and microbes. Organic acids were easily anaerobically converted through acetogenesis and methanogenesis, but acetogenesis was inhibited by the increased concentrations of potential inhibitors (N-heterocycles and aromatic compounds). Nitrogen containing polymers could be degraded into smaller organics but not be further mineralized. Ozone pretreatment promoted the conversion of organics and methane production by oxidizing inhibitors and enhancing the degradation of the macromolecules into low molecular weight chemicals. However, these compounds were not further converted by microbes or effectively adsorbed by GAC. An increase of the microbial lag-phase in repeated experiments indicated the drawback of the combination of ozone pretreatment and anaerobic conversion. Souza *et al.* reported that the toxicity of wastewater containing azo dyes increased as a consequence of ozonation.[56] Another concern was that the ozone dosage in the pretreatment process (2.1 mg O_3 per mL HTL aqueous phase) would significantly increase the cost. In general, GAC addition showed promising application prospects as its buffer ability for inhibitors and enrichment of microbes which enhanced the degradation of organic acids and potential inhibitors. In addition, GAC adsorbed recalcitrant organics degraded from nitrogen containing polymers by the microbes. Hence, a COD removal of 93.3–96.8% was achieved. Considering the potential commercial applications, GAC may be packed in a high rate reactor to act as a biofilm carrier to enhance the conversion of inhibitors and acids at the same time. However, a decrease of the conversion of organics may happen throughout the long-term operation of the reactor because about 30% of the organics in the HTL aqueous phase are recalcitrant. It is therefore difficult to biologically regenerate. The development of activated carbon from cheap sources (biowaste) to replace costly commercial GAC is another promising direction.[57] Hydrochar produced from the HTL process has been proposed as a low-cost adsorbent,[58,59] but needs further evaluation.

Most organics (93.3–96.8%) including toxic compounds in the HTL aqueous phase were converted through strengthened anaerobic conversion. The effluent of anaerobic conversion with polished nitrogen (71–73% ammonia) could be used as a nutrient source for algae cultivation (Fig. 6), and the produced biomass could be fed back to the HTL process. Hence, a multi-cycle nutrient reuse for multiple cycles of an algal biomass and bioenergy production system could be built. Zhou *et al.* proved that the Environment-Enhancing Energy system could significantly increase the energy output by directly recycling

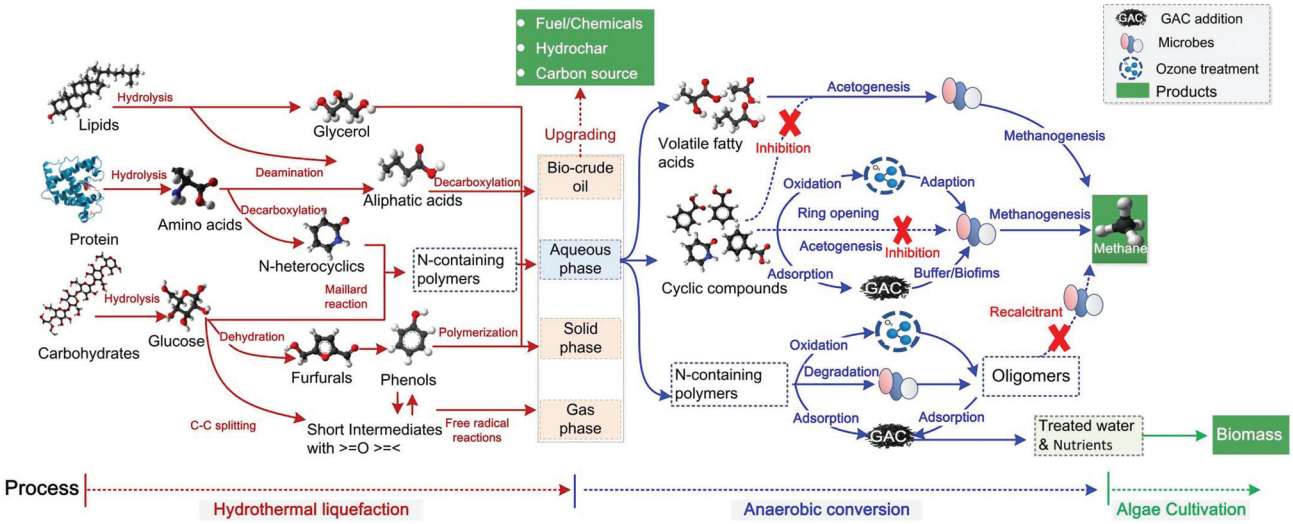

Fig. 6 Biorefinery roadmap of waste biomass through combined HTL and enhanced anaerobic conversion. (HTL pathway was modified based on Toor *et al.*,[55] and Kumar *et al.*[1]).

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the HTL aqueous phase *via* algae cultivation.[7] By combining with anaerobic conversion, the Environment-Enhancing Energy system could be further improved in three primary aspects: firstly, the complete conversion of organics during anaerobic conversion would avoid the risk of discharging toxic compounds into the environment. Secondly, methane production during anaerobic conversion could further improve the energy recovery of the whole process. Over 70% of organics in the HTL aqueous phase could be recovered in the form of biomethane through anaerobic conversion. Thirdly, anaerobic conversion could act as a detoxification and nutrient polishing step for algae cultivation, which has proven to increase the biomass yield and the production rate and decrease the dilution ratio for the HTL aqueous phase.[60]

Combining HTL and catalytic hydrothermal gasification (CHG) is also promising, in which a 90% conversion of organics in the HTL aqueous phase along with a production of hydrogen rich gas was achieved.[61–63] However, CHG operates at high temperatures (400–700 °C) and often requires catalysts. According to the U.S. Department of Energy's report,[64] the cost of CHG conversion of the HTL aqueous phase was 44% of the total cost (excluding the feedstock cost). The separation of value-added chemicals such as acids, sugars and phenols from the HTL aqueous phase have also been recently reported.[65] However, the separation currently accounts for 60 to 80% of the process cost of most mature chemical industry processes.[66] Hence, the separation and concentration processes make it not economically viable at an industrial scale. In comparison, waste biorefinery *via* combined HTL and intensified anaerobic conversion is sustainable and economically competitive. This should be attributed to its mild reaction conditions and enhanced energy recovery.

Conclusion

This study indicated that over 30% of the organics in the HTL aqueous phase were unable to be converted by anaerobic conversion. MALDI-TOF-MS analysis indicated that the remaining organics may be oligomers (100–300 Da) which were generated from nitrogen containing polymers (Da >1000). Methane production was mostly attributed to the conversion of organic acids. The inhibition of acetogenesis caused by potential inhibitors was observed with the increase of the HTL aqueous phase strength (>10 g COD per L). Ozone pretreatment could improve the conversion of organics, methane production rate and methane yield at high concentrations of the HTL aqueous phase (10–20 g COD per L) by converting the inhibitors before anaerobic conversion. However, an increase of the lag-phase was observed and products (500–1000 Da) from nitrogen containing macromolecules were neither adsorbed by GAC nor converted by microbes. GAC addition resulted in an improvement of the methane yield by 298% at 2 dilution rates of the HTL aqueous phase. The repeated experiment showed a further decrease of the lag-phase and an enhancement of the conversion of acids in the GAC added group due to the formation of biofilms. Illumina MiSeq sequencing revealed the enrichment of the detoxification bacteria Anaerolineaceae, Burkholderiaceae, and Peptococcaceae and also syntrophic acetogens on GAC. As for the methanogens, the biofilm content of Methanomicrobiaceae and Methanosarcinaceae all demonstrated a notable increase, which is believed to be related to strengthened acetogenesis and acetate conversion. In addition, the GAC addition could adsorb the recalcitrant oligomers, which further enhanced the COD removal (93.3–96.8%). Hence, the intensified anaerobic conversion could enhance the energy and nutrient recovery in the whole HTL process, which also improves the feasibility of commercializing HTL technology.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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References

1 G. Kumar, S. Shobana, W. Chen, Q. Bach, S. H. Kim, A. E. Atabani and J. Chang, *Green Chem.*, 2017, **19**, 44–67.
2 A. R. K. Gollakota, N. Kishore and S. Gu, *Renewable Sustainable Energy Rev.*, 2018, **81**, 1378–1392.
3 R. Posmanik, C. M. Martinez, B. Cantero-Tubilla, D. A. Cantero, D. L. Sills, M. J. Cocero and J. W. Tester, *ACS Sustainable Chem. Eng.*, 2017, **6**, 2724–2732.
4 T. H. Pedersen, C. U. Jensen, L. Sandström and L. A. Rosendahl, *Appl. Energy*, 2017, **202**, 408–419.
5 B. Zhao, Z. Wang, Z. Liu and X. Yang, *Green Chem.*, 2016, **18**, 5254–5265.
6 G. Yu, Y. Zhang, L. Schideman, T. Funk and Z. Wang, *Energy Environ. Sci.*, 2011, **4**, 4587–4595.
7 Y. Zhou, L. Schideman, G. Yu and Y. Zhang, *Energy Environ. Sci.*, 2013, **6**, 3765–3779.
8 C. M. Godwin, D. C. Hietala, A. R. Lashaway, A. Narwani, P. E. Savage and B. J. Cardinale, *Environ. Sci. Technol.*, 2017, **51**, 11450–11458.
9 Y. Li, S. Leow, A. C. Fedders, B. K. Sharma, J. S. Guest and T. J. Strathmann, *Green Chem.*, 2017, **19**, 1163–1174.
10 M. Zheng, L. C. Schideman, G. Tommaso, W. Chen, Y. Zhou, K. Nair, W. Qian, Y. Zhang and K. Wang, *Energy Convers. Manage.*, 2017, **141**, 420–428.
11 G. Tommaso, W. Chen, P. Li, L. Schideman and Y. Zhang, *Bioresour. Technol.*, 2015, **178**, 139–146.

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12 Y. Zhou, L. Schideman, M. Zheng, A. Martin-Ryals, P. Li, G. Tommaso and Y. Zhang, *Water Sci. Technol.*, 2015, **72**, 2139–2147.
13 H. Chen, C. Zhang, Y. Rao, Y. Jing, G. Luo and S. Zhang, *Biotechnol. Biofuels*, 2017, **10**, 140–156.
14 Y. Zheng, J. Zhao, F. Xu and Y. Li, *Prog. Energy Combust. Sci.*, 2014, **42**, 35–53.
15 N. Sudasinghe, B. Dungan, P. Lammers, K. Albrecht, D. Elliott, R. Hallen and T. Schaub, *Fuel*, 2014, **119**, 47–56.
16 Z. Zhu, Z. Liu, Y. Zhang, B. Li, H. Lu, N. Duan, B. Si, R. Shen and J. Lu, *Bioresour. Technol.*, 2016, **199**, 220–227.
17 B. Maddi, E. Panisko, T. Wietsma, T. Lemmon, M. Swita, K. Albrecht and D. Howe, *Biomass Bioenergy*, 2016, **93**, 122–130.
18 B. Maddi, E. Panisko, T. Wietsma, T. Lemmon, M. Swita, K. Albrecht and D. Howe, *ACS Sustainable Chem. Eng.*, 2017, **5**, 2205–2214.
19 B. Si, J. Li, Z. Zhu, M. Shen, J. Lu, N. Duan, Y. Zhang, Q. Liao, Y. Huang and Z. Liu, *Sci. Total Environ.*, 2018, **630**, 1124–1132.
20 S. R. Shanmugam, S. Adhikari and R. Shakya, *Bioresour. Technol.*, 2017, **230**, 43–48.
21 S. R. Shanmugam, S. Adhikari, Z. Wang and R. Shakya, *Bioresour. Technol.*, 2017, **223**, 115–120.
22 O. Gibert, B. T. Lefèvre, M. Fernández, X. Bernat, M. Paraira, M. Calderer and X. Martínez-Lladó, *Water Res.*, 2013, **47**, 1101–1110.
23 L. Bertin, S. Berselli, F. Fava, M. Petrangeli-Papini and L. Marchetti, *Water Res.*, 2004, **38**, 3167–3178.
24 U. Hübner, U. von Gunten and M. Jekel, *Water Res.*, 2015, **68**, 150–170.
25 M. C. Valsania, F. Fasano, S. D. Richardson and M. Vincenti, *Water Res.*, 2012, **46**, 2795–2804.
26 O. Chedeville, M. Debacq and C. Porte, *Desalination*, 2009, **249**, 865–869.
27 L. Yang, B. Si, M. A. Martins, J. Watson, H. Chu, Y. Zhang, X. Tan, X. Zhou and Y. Zhang, *Water Sci. Technol.*, 2018, t2018108.
28 B. Si, Z. Liu, Y. Zhang, J. Li, R. Shen, Z. Zhu and X. Xing, *Int. J. Hydrogen Energy*, 2016, **41**, 4429–4438.
29 J. S. Martinez-Fernandez and S. Chen, *Algal Res.*, 2017, **25**, 274–284.
30 L. Chen, T. Zhu, J. S. M. Fernandez, S. Chen and D. Li, *Algal Res.*, 2017, **27**, 311–317.
31 B. E. Rittmann and P. L. McCarty, *Environmental biotechnology: principles and application*, McGraw-Hill, 2001.
32 S. G. Pavlostathis, *Crit. Rev. Environ. Control*, 1991, **21**, 411–490.
33 APHA, AWWA and WEF, *Standard methods for the examination of water and wastewater*, United Book Press, 1998.
34 H. Li, Z. Liu, Y. Zhang, B. Li, H. Lu, N. Duan, M. Liu, Z. Zhu and B. Si, *Bioresour. Technol.*, 2014, **154**, 322–329.
35 Z. Zhu, B. Si, J. Lu, J. Watson, Y. Zhang and Z. Liu, *Bioresour. Technol.*, 2017, **243**, 9–16.
36 M. Pham, L. Schideman, J. Scott, N. Rajagopalan and M. J. Plewa, *Environ. Sci. Technol.*, 2013, **47**, 2131–2138.
37 L. Leng, J. Li, Z. Wen and W. Zhou, *Bioresour. Technol.*, 2018, **256**, 529–542.
38 H. Yao, Y. Ren, X. Deng and C. Wei, *J. Hazard. Mater.*, 2011, **186**, 1136–1140.
39 H. Chen, J. Wan, K. Chen, G. Luo, J. Fan, J. Clark and S. Zhang, *Water Res.*, 2016, **106**, 98–107.
40 D. López Barreiro, M. Bauer, U. Hornung, C. Posten, A. Kruse and W. Prins, *Algal Res.*, 2015, **9**, 99–106.
41 M. Déniel, G. Haarlemmer, A. Roubaud, E. Weiss-Hortala and J. Fages, *Energy Fuels*, 2016, **30**, 4895–4904.
42 R. Posmanik, R. A. Labatut, A. H. Kim, J. G. Usack, J. W. Tester and L. T. Angenent, *Bioresour. Technol.*, 2017, **233**, 134–143.
43 A. A. Peterson, R. P. Lachance and J. W. Tester, *Ind. Eng. Chem. Res.*, 2010, **49**, 2107–2117.
44 C. Zhang, X. Tang, L. Sheng and X. Yang, *Green Chem.*, 2016, **18**, 2542–2553.
45 C. Kunacheva and D. C. Stuckey, *Water Res.*, 2014, **61**, 1–18.
46 G. Montaudo, F. Samperi and M. S. Montaudo, *Prog. Polym. Sci.*, 2006, **31**, 277–357.
47 M. Tuomela, M. Vikman, A. Hatakka and M. Itävaara, *Bioresour. Technol.*, 2000, **72**, 169–183.
48 T. A. Kurniawan, W. Lo and G. Y. S. Chan, *J. Hazard. Mater.*, 2006, **137**, 443–455.
49 L. Bijan and M. Mohseni, *Water Res.*, 2005, **39**, 3763–3772.
50 F. Rosenkranz, L. Cabrol, M. Carballa, A. Donoso-Bravo, L. Cruz, G. Ruiz-Filippi, R. Chamy and J. M. Lema, *Water Res.*, 2013, **47**, 6739–6749.
51 E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt and F. Thompson, in *The Prokaryotes*, Springer, 2014.
52 B. Si, J. Li, Z. Zhu, Y. Zhang, J. Lu, R. Shen, C. Zhang, X. Xing and Z. Liu, *Biotechnol. Biofuels*, 2016, **9**, 254–269.
53 J. R. Sieber, M. J. McInerney and R. P. Gunsalus, *Annu. Rev. Microbiol.*, 2012, **66**, 429–452.
54 J. Ma, B. Zhao, C. Frear, Q. Zhao, L. Yu, X. Li and S. Chen, *Bioresour. Technol.*, 2013, **137**, 41–50.
55 S. S. Toor, L. Rosendahl and A. Rudolf, *Energy*, 2011, **36**, 2328–2342.
56 S. Souza, K. Bonilla and A. Souza, *J. Hazard. Mater.*, 2010, **179**, 35–42.
57 S. Wong, N. Ngadi, I. M. Inuwa and O. Hassan, *J. Cleaner Prod.*, 2018, **175**, 361–375.
58 L. Cao, C. Zhang, H. Chen, D. C. W. Tsang, G. Luo, S. Zhang and J. Chen, *Bioresour. Technol.*, 2017, **245**, 1184–1193.
59 C. Falco, N. Baccile and M. Titirici, *Green Chem.*, 2011, **13**, 3273.
60 L. Yang, B. Si, X. Tan, H. Chu, X. Zhou, Y. Zhang, Y. Zhang and F. Zhao, *Bioresour. Technol.*, 2018, **266**, 349–356.
61 J. Watson, B. Si, H. Li, Z. Liu and Y. Zhang, *Int. J. Hydrogen Energy*, 2017, **42**, 20503–20511.
62 R. Cherad, J. A. Onwudili, P. Biller, P. T. Williams and A. B. Ross, *Fuel*, 2016, **166**, 24–28.
63 L. Zhang, P. Champagne and C. Charles Xu, *Bioresour. Technol.*, 2011, **102**, 8279–8287.

64 Bioenergy Technologies Office, *Multi-Year Program Plan*, US Departmen of Energy, 2016.
65 H. Lyu, K. Chen, X. Yang, R. Younas, X. Zhu, G. Luo, S. Zhang and J. Chen, *Sep. Purif. Technol.*, 2015, **147**, 276–283.
66 A. J. Ragauskas, C. K. Williams, B. H. Davison, G. Britovsek, J. Cairney, C. A. Eckert, W. J. Frederick, J. P. Hallett, D. J. Leak, C. L. Liotta, J. R. Mielenz, R. Murphy, R. Templer and T. Tschaplinski, *Science*, 2006, **311**, 484–489.

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