Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. 2017, 51, 12683-12691

pubs.acs.org/est

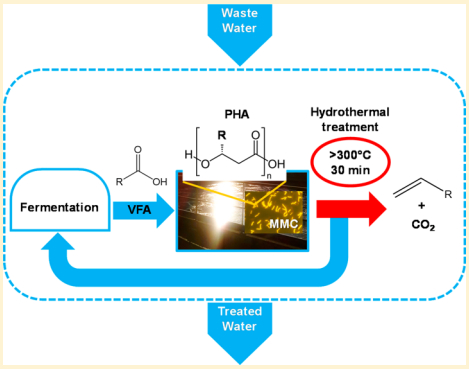
Renewable Alkenes from the Hydrothermal Treatment of Polyhydroxyalkanoates-Containing Sludge

Cristian Torri,*[,†] Tom Detert Oude Weme,[‡] Chiara Samorì,[†] Alisar Kiwan,[†] and Derk W. F. Brilman*[,‡]

[†]Laboratori "R. Sartori", Dipartimento di Chimica "Giacomo Ciamician", Università di Bologna, Via Sant'Alberto 163, 48123, Ravenna, Italy

[‡]Sustainable Process Technology Group, Faculty of Science and Technology, University of Twente, P.O. Box 217, 7500AE Enschede, The Netherlands

ABSTRACT: Polyhydroxyalkanoates (PHA) are a key constituent of excess sludge produced by Aerobic Sewage Sludge Treatment plants. The accumulation of significant amount of PHA inside aerobic microbial cells occurs when a surplus of an easily degradable carbon source (e.g., volatile fatty acids, VFA) is found in combination with other nutrients limitation. Herein, hydrothermal treatment (HT) of PHA-containing sludge at 300 and 375 °C was demonstrated to be effective in converting most (>70% w/w) of the bacterial PHA stored inside microbial cells into alkene/CO_2 gas mixtures. Simultaneously, most of non-PHA biomass was converted into water-soluble compounds (50% carbon yield) that were acidogenic fermented to produce volatile fatty acids, ideal substrate to feed aerobic bacteria and produce more PHA. According to results here presented, HT of excess sludge with moderate (13%) PHA content can produce about 50 kg of alkenes per tonne of suspended solids treated, with a significant reduction of sludge mass (80% reduction of wet sludge volume) and consequent disposal cost.



INTRODUCTION

Aerobic Sewage Sludge Treatment (SST) is one of the most common technology for wastewater treatment. SST of domestic wastewater produces about 20−30 kg y^{-1} of excess sludge (ES) on dry basis per person, corresponding to a worldwide potential of more than 100 Mt y^{-1}.[1] Most of this amount is currently disposed at an average cost of 40−120 €/dry tonne, according to the disposal strategy. In the best practices, the sludge is anaerobically digested to biogas (removing up to 30% volatile solids content),[2] applied on soil, dried and incinerated, or landfilled.[3−5] The mixed microbial communities (MMC) that are involved in SST and constitute most of ES mass, convert the soluble organics into suspended solids (e.g., living microorganisms), often accumulating polyhydroxyalkanoates (PHA) as storage material.[6] Specifically, the accumulation of PHA inside bacterial cells occurs when the carbon source is in excess, but one or several other nutrients are limited. In MMC, the relative proportion of PHA-producing microorganisms can be enhanced if the bacteria are subjected to pulsed feed of easily biodegradable substances, using the so-called "feast and famine regime" or uncoupled carbon and nutrient availabilities.[7−9] In principle, these conditions can be found in the SST plants due to dynamic organic load regime (e.g., variable organic loading rate or change in wastewater discharge rate). As result, if ES coming from SST is fed with easily degradable carbon substrates (e.g., volatile fatty acids, VFA), it can accumulate from 5% up to 30% dry weight of PHA.[10,11] PHA content in ES depends on the process structure or sludge age and can become very significant (>40% $w/w_{dried\ sludge}$) if the sludge is fed with easily degradable wastewater with high C/N ratio (e.g., fermented wastewater from food manufacturing).[12] PHA accumulation capability of the bacterial sludge can even reach PHA content up to 60% w/w by enhancing the feast and famine regime to select PHA-accumulating organisms in an optimized SST.[9] This concept was recently applied at pilot scale by Morgan-Sagastume et al.: they demonstrated the feasibility of PHA production by feeding an ES produced under feast and famine conditions with VFA generated from the ES fermentation itself.[13] This relatively simple and reliable process opens the possibility of converting a relevant portion of various water-soluble substances into a chemically defined material (PHA) that can be extracted[14] and used as source of chemicals and bioplastics.[15]

A complete and selective extraction of PHA from MMC is challenging and often compromises the quality of the polymer itself.[16] An alternative, and relatively unexplored approach is the conversion of PHA into chemicals of interest by treating the whole biomass through thermochemical treatment. Whereas hydrolysis, alcoholysis and thermolysis of PHA can produce 2-hydroxyalkanoic acids,[17] 2-hydroxyalkanoic esters[18] and 2-alkenoic acids,[19,20] respectively, recent studies have shown that

Received: July 31, 2017
Revised: September 30, 2017
Accepted: October 9, 2017
Published: October 9, 2017

DOI: 10.1021/acs.est.7b03927
Environ. Sci. Technol. 2017, 51, 12683−12691

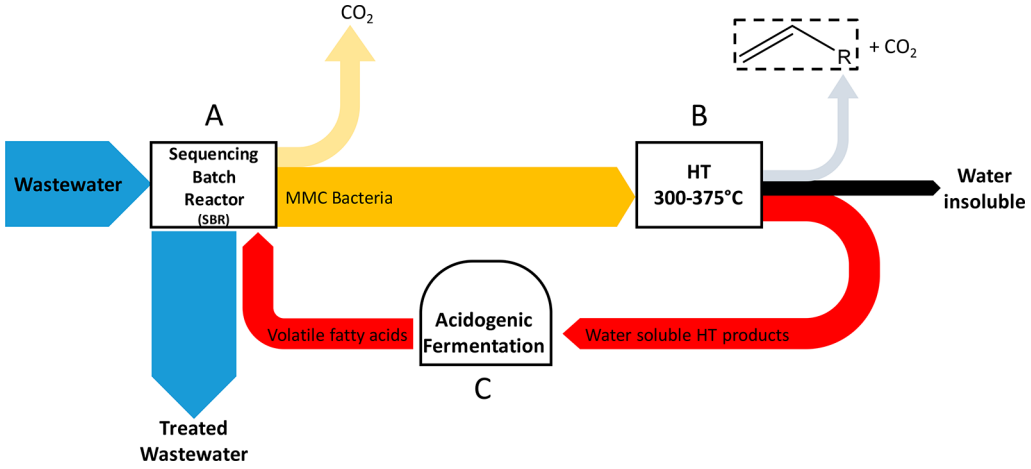


Figure 1. overall wastewater-to-alkenes concept investigated in the present paper. A: sequencing batch reactor operated with feast-and-famine pulsed feed; B: hydrothermal treatment reactor; C: short residence time acidogenic fermenter for the conversion of aqueous products from HT (HTW) into VFA.

hydrothermal treatment (HT) could provide a high yield of short 1-alkenes that can be used as drop-in chemicals.[21] Additionally, HT of non-PHA biomass yields variable amount of water insoluble substances (solids and oily fraction) and water-soluble products (hydrothermal treatment aqueous product, HTW), with a consequent solubilization of a large portion of the whole organic matter.[22−28]

Several authors demonstrated that hydrothermal pretreatment increases the sludge solubilization, with a larger effect under more severe conditions.[29] HT at moderate temperature (<200 °C) typically increases ES anaerobic biodegradability toward methane or VFA,[29−31] whereas a HT temperature higher than 220 °C is usually considered detrimental to anaerobic consortia under standard conditions of anaerobic digestion, since this harsher treatment can produce potentially toxic compounds.[32,33] Recent works on HT of microalgae at high temperature showed that, if toxicity effect is attenuated by dilution, HTW can be anaerobically converted with acceptable yields.[34] On the other hand, without a large dilution or pretreatment, HTW totally inhibit the methanogenic activity. The effect of HTW on acidogenic consortia is less known; Si et al.[35] recently reported that relatively diluted HTW coming from HT of lignocellulose feedstock at 260 °C can be converted into VFAs (subsequently converted to methane in a second stage) with high yield and without a significant inhibition of the acidogenesis.

The present paper aims at evaluating the potential of a new "wastewater-to-propylene route" (Figure 1). In this concept, MMC are fed with VFA to obtain PHA enriched sludge (A). Then this sludge is converted by HT into gaseous alkenes, water-soluble substances, and a solid residue (B). The water-soluble substances are fermented into a VFA enriched solution that can be again used to feed MMC (C). HT yield and composition, and acidogenic fermentation of hydrothermal treatment aqueous products are evaluated for different HT conditions to assess the feasibility of the proposed concept.

MATERIALS AND METHODS

Chemicals. All solvents and chemicals used in this study were obtained from Sigma-Aldrich (purities ≥98%) and were used without purification. Standard polyhydroxybutyrate (PHB) was purchased from Biomer (DE).

Microbial Mixed Culture (MMC) Production and Characterization. A continuous lab-scale sequencing batch reactor (SBR, 5 L working volume) was built and operated as shown by Villano et al. (2014) with slight modifications.[14] The reactor was inoculated in the early 2015 with an activated sludge from a municipal wastewater treatment plant (located in Ravenna, Italy) with stable operations after 12 months. PHA enriched biomass was obtained after 3 months from the reaching of SBR stable operations. The culture was fed with a synthetic VFA solution that simulates a fermented wastewater, containing mineral medium and VFA (overall concentration of 4.31 g COD L^{-1}) in the following concentrations: acetic acid 1.8 g L^{-1}, propionic acid 0.2 g L^{-1}, isobutyric acid 0.1 g L^{-1}, butyric acid 0.6 g L^{-1}, isovaleric acid 0.3 g L^{-1}, valeric acid 0.1 g L^{-1}. The following mineral medium composition was prepared per liter of tap water: 2 g NaOH, 80 mg NH_4Cl, 10 mg $MgSO_4 \cdot 7H_2O$, 100 mg EDTA, 9 mg K_2HPO_4, 20 mg KH_2PO_4, 70 mg $CaCl_2 \cdot 2H_2O$ and 3 mL of trace element solution. The trace element solution consisted of (per liter of distilled water): 1500 mg $FeCl_3 \cdot 6H_2O$, 150 mg H_3BO_3, 150 mg $CoCl_2 \cdot 6H_2O$, 120 mg $MnCl_2 \cdot 4H_2O$, 120 mg $ZnSO_4 \cdot 7H_2O$, 60 mg $Na_2MoO_4 \cdot 2H_2O$, 30 mg $CuSO_4 \cdot 5H_2O$ and 30 mg of KI.

The length of the SBR cycle was 6 h (4 cycles per day) according to Villano et al. (2014). During all operations, the SBR was mixed with an aquarium submersible pump (water discharge of 10 L min^{-1}) and bubbled (3 L min^{-1} air) with an air stone placed on the bottom of the reactor. Each cycle consisted of an initial feeding phase and an aerobic reaction phase. During the feeding, 1 L of synthetic wastewater displaces 1 L of reactor content to the accumulation reactor. Then the slurry is aerated for 1 h (enough to complete the feast phase) before collection, filtration and freeze-drying. This configuration typically yielded 1.5−2 g L^{-1} of bacterial biomass with 10−15% $w_{PHA}/w_{dry\text{-}biomass}$. PHA accumulated inside bacterial cells was a poly(hydroxybutyrate-*co*-hydroxyvalerate) copolymer (P(HB-HV)), with HB/HV mass ratio of 90/10 (92/8 molar ratio). A mix of similar samples collected from several days of SBR operation was mixed, homogenized, and analyzed to get a suitable amount of freeze-dried biomass for all HT tests starting from the same sample. The PHA content, determined by mean of previously published method,[19] of homogenized sample was 13.3 ± 2.5% w_{PHA}/w_{dry} (mean ± standard deviation, n = 10). This bulk sample had the following

DOI: 10.1021/acs.est.7b03927
Environ. Sci. Technol. 2017, 51, 12683−12691

characteristics: 36% C, 4.8% N, 5.2% H, 1.5% S, 40% O (determined as shown in the Characterization of HT Products and Fermented HTW section), and 12% ash (by calcination at 550 °C) with theoretical chemical oxygen demand (COD) of 1.00 gO_2/g_{dry} (determined by stoichiometry from elemental analysis).

Hydrothermal Treatment of PHB and PHA-Containing Biomass. A stainless-steel reactor (45 mL) was loaded with PHA-containing biomass or PHB (2 g) and water (18 g), closed and purged twice with 100 bar of N_2. Pressure was then adjusted to 7.5 bar and the bacterial suspension was stirred for 5 min before the beginning of the experiment. The different temperatures tested in each HT experiment were kept by keeping the reactor in a heated and fluidized sand bath. At the end of the experiment, gas was collected through a glass gasometer filled with slightly acidic water, quantified by the volume of water displaced and analyzed. GC-TCD Analysis of gas from HT by gas chromatography was performed with a Varian-450 micro-GC system with three parallel channels for analysis of H_2, other permanent gas (e.g., CO and CO2) and hydrocarbons.

After degassing, an aqueous slurry and some water-insoluble droplets attached to the walls were found inside the reactor. The aqueous slurry was poured from the reactor and centrifuged, yielding: (i) an aqueous phase (thereafter called HTW), analyzed by GC-MS and elemental analysis (see below for details), and used for further fermentation tests; and (ii) a wet solid residue. The water-insoluble droplets attached to the walls were dissolved in acetone, put together with the wet solid residue and dried overnight, yielding a water-insoluble residue (thereafter called water insoluble product) that was analyzed by elemental analysis (see below details). The typical product collection and characterization scheme is shown in Figure 2.

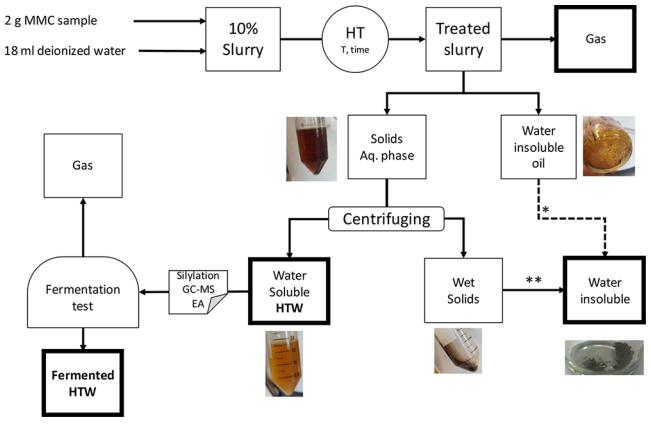


Figure 2. experimental scheme and chemical characterization of HT products. *mixing with wet solid and overnight drying; **overnight drying.

Fermentation of HTW. In preliminary HTW acidogenic fermentation test, 100 mL syringe reactors were used, according to a modified version of Hohenheimer Biogas Yield Test previously developed.[36]

The inoculum for acidogenic fermentation tests, taken from an anaerobic digester treating ES, had the following characteristics:

- Total Suspended solids (TSS): 56 g L^{-1};
- Volatile Suspended Solids (VSS): 35 g L^{-1};
- COD: 45 gO_2 L^{-1}.

The acidogenic fermentation tests were performed in both batch and semicontinuous mode.

In the batch experiments, inoculum (19 mL) was autoclaved at 122 °C (to remove methanogens) for 1 h and then transferred into the syringe reactor together with HTW (1 mL) obtained at 300 and 375 °C for 30 min. According to the HTW composition, this corresponds to a final concentration of organics equal to 2.05 g L^{-1} (300 °C) and 1.85 g L^{-1} (375 °C) of HTW derived organics. A blank reactor was prepared in the same way but without HTW. VFA concentration was monitored every 5−6 days for 20 days. The amount of VFA from HTW was calculated by subtracting the VFA concentration observed in the blank from the VFA concentration observed in the reactor fed with HTW. VFA yield was obtained by dividing VFA amount by the concentration of HTW organics added (2.05 g L^{-1} and 1.85 g L^{-1} respectively for 300 and 375 °C HTW).

In the semicontinuous experiments, the same system was operated as an anaerobic sequencing batch reactor (SBR) by addition of liquid and withdrawal of liquid digestate (separated from solid bacterial biomass). Every 3 days, part of the reactor content (8−10 mL) was withdrawn and centrifuged to separate solids and liquid digestate. An aliquot of the fermented liquid (6 mL) was removed and stored for GC-MS analysis (with or without derivatization) and elemental analysis (see below), whereas centrifuged solids and the remaining liquid were mixed with fresh liquid feed (6 mL of glucose or HTW) with a vortex and put back in the syringe reactor. In system set up, 2 mL d^{-1} of 20 g L^{-1} glucose solution was added for 10 days, producing spontaneous acidification, and reaching a value of 12−14 g L^{-1} of VFA. After 10 days, the system was fed with 2 mL d^{-1} of HTW (obtained from HT at 300 and 375 °C for 30 min), corresponding to a hydraulic retention time (HRT) of 10 days (organic loading rate, OLR, of 1.5 g L^{-1} d^{-1}).

Characterization of HT Products and Fermented HTW. Nonvolatile water-soluble products in HTW were quantified by BRIX densimetry as previously described by Oasmaa and Kuoppala.[37]

Elemental analysis (C, H, and N) was performed on lyophilized bacterial biomass (2−3 mg), water insoluble solids (2−3 mg), HTW (25 mg) and fermented HTW (25 mg) using a Thermo Fisher organic elemental analyzer (Flash 2000) configured for solid samples with a copper/copper oxide column and calibrated with 2,5-Bis(5-*tert*-butyl-2-benzo-oxazol-2-yl) thiophene (BBOT). The HTW and fermented HTW were analyzed by adsorbing 25 μL of sample on alumina power (25 mg), used as support for minimizing volatile compound losses during sample loading. Ammonia analysis was performed spectrophotometrically through the Nessler colorimetric method (method 8038, USEPA). Briefly, after dilution with demineralized water, the sample was supplemented with three drops of Mineral Stabilizer, three drops of Poly(vinyl alcohol) and 1 mL of Nessler reactive, in a reaction volume of 25 mL. After shaking, the absorbance was read at 425 nm. The GC-MS characterization of VFA and other soluble GC-MS detectable compounds was performed by direct analysis, and after water evaporation and derivatization. Methods previously published were slightly modified according to further improvements applied in the time.[36,38] For VFA analysis, an aliquot of the sample (0.1 mL of HTW or fermented HTW) was added to 2-ethylbutyric acid solution in H_2O (internal standard at 1000 ppm, 0.1 mL), saturated $KHSO_4$ solution (0.1 mL), NaCl brine (0.1 mL) and dimethyl carbonate (1 mL). The biphasic

DOI: 10.1021/acs.est.7b03927
Environ. Sci. Technol. 2017, 51, 12683−12691

Table 1. Mass Yield (w/w %) of Products from HT of Pure PHB at Different Reaction Conditions

temperature	°C	300	300	300	325	325	325	350	375
time	min.	10	20	30	10	20	30	20	20
CO_2	% w/w	12	21	30	23	37	40	39	42
propene	% w/w	15	21	28	24	34	36	39	40
other gases	% w/w	0.11	0.02	0.02	0.07	0.02	0.16	0.22	0.43
acetic acid	% w/w	0.46	0.44	0.74	0.56	0.81	0.81	0.98	4.09
propanoic acid	% w/w	0.09	0.09	0.07	0.05	0.01	0.01	0.01	0.06
butanoic acid	% w/w	0.08	0.09	0.06	0.04	0.03	0.00	0.05	0.06
(z)-2-butenoic acid	% w/w	3.3	3.3	2.5	3.1	1.8	1.1	0.60	0.08
3-butenoic acid	% w/w	1.7	1.5	1.2	1.6	0.8	0.4	0.25	0.16
(e)-2-butenoic acid	% w/w	8.9	8.7	6.4	7.4	5.5	4.3	2.6	0.78
2-hydroxy-butanoic acid	% w/w	0.9							

solution was shaken and the upper phase, containing VFA dissolved in dimethyl carbonate, was analyzed by GC−MS. GC−MS analysis of VFA was performed by using a 7820A Agilent HP gas chromatograph connected to a 5977E Agilent HP quadrupole mass spectrometer. The injection port temperature was 250 °C. Analytes were separated by a DB-FFAP capillary column (nitroterephthalic-acid-modified polyethylene glycol, 30 m, 0.25 mm i.d., 0.25 μm film thickness), with helium as carrier gas (at constant pressure, 33 cm s^{-1} linear velocity at 200 °C). Mass spectra were recorded under electron ionization (70 eV) at a frequency of 1 scan s^{-1} within the 33−600 m/z range. The following thermal program was used: from 50 °C (held for 5 min) to 250 °C (held for 12 min) at 10 °C min^{-1}. VFA calibration was performed by applying the same procedure to standard solutions containing known amounts of the three VFA analyzed (acetic, propionic, and butyric acid).

The quantitation of GC-MS detectable organics in HTW was performed after water evaporation and derivatization. An aliquot of HTW (0.1 mL) was spiked with sorbitol solution (0.1 mL of 200 mg L^{-1} solution used as internal standard) and dried under nitrogen flow at 60 °C. Subsequently, bis-trimethylsilyl-trifluoroacetamide with 1% trimethyltrichlorosilane (0.2 mL) and acetonitrile (0.1 mL) were added to the dried residue and heated at 60 °C for 1 h to obtain the complete derivatization of cyclic dipeptides. Subsequently, to complete the derivatization of polyhydroxylated compounds, a drop of pyridine (0.01 mL) was added and the sample was heated for 1 h. The solution was finally diluted in ethyl acetate (0.5 mL) and analyzed by GC-MS. The analysis was performed at 280 °C in splitless mode in the injection port of a 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer. Analytes were separated by a MDN-5S (Supelco) fused-silica capillary column (stationary phase poly[5% diphenyl/95% dimethyl]siloxane, 30 m, 0.25 mm i.d., 0.25 μm film thickness) using helium as carrier gas (at constant pressure, 33 cm s^{-1} linear velocity at 200 °C). Mass spectra were recorded under electron ionization (70 eV) at a frequency of 1 scan s^{-1} within the m/z 12−950 Da range. The following thermal program was used: 50 °C for 5 min followed by temperature increase from 50 to 325 at 10 °C/min. Compounds identification was performed by means of MS libraries, and by comparison with previously published spectra or with internal laboratory database. Quantitation was performed by assuming the unitary response factor with respect to internal standard (sorbitol).

RESULTS AND DISCUSSION

HT of Pure PHB. An initial set of HT tests was performed on pure PHB to select the best reaction conditions (temperature and time) and optimize PHA-to-propene conversion. Each tested condition gave a significant propene/CO_2 production and a variable amount of PHB degradation intermediates (Table 1). In all tests, the main products were propene and CO_2, with molar ratio close to 1:1, suggesting an intrinsic high selectivity of the reaction. The gas composition revealed an extraordinary selectivity toward propene/CO_2, with minimal (<1% w/w) yield of other gaseous products (mainly CO and CH_4). Semivolatile intermediates derived from PHB-thermal decomposition, namely 2-alkenoic and 3-alkenoic acids, were detected in the aqueous fraction, especially at lower temperature and time. On the other hand, products coming from PHB hydrolysis, namely 2-hydroxyacids, were not detected in all conditions but 300 °C for 10 min (0.9% w/w_{PHB}), suggesting that the predominating reaction under HT was PHB thermolysis and not PHB hydrolysis. The achieved results were in good agreement with experimental results of Wagner et al, who observed 35−75% molar yield of propene/CO_2 from the HT of pure PHB at 300−360 °C range.[26] Moreover, the detailed GC-MS analysis here presented suggested that under HT at temperature higher than 300 °C, depolymerization/decarboxylation was more favored than depolymerization or hydrolysis, as testified by the total absence of β-hydroxyacids in most of the experimental conditions tested.

HT of PHA-Containing MMC. After preliminary tests on pure PHB, HT was performed on MMC biomass with a PHA content of 13.3 ± 2.5% w_{PHA}/w_{dry}, typical value for wastewater treatment with activated sludge. HT was performed on 10% $w_{dry}/w_{solution}$ MMC bacterial slurry to simulate a biomass concentration easily achievable with solid/liquid separation for a broad range of ES.[39] Based on preliminary test on pure PHB, two temperatures (300 and 375 °C), and five reaction times (7,10,15,20 and 30 min) were applied to understand the process and identify the most suitable reaction conditions.

Under all investigated conditions, alkenes (mainly propene and 1-butene) and CO_2 were the main constituents of HT gas, in agreement with Wagner et al.[21] data on PHB-containing cyanobacteria. The relative amount of propene and 1-butene approached the mass ratio of HB and HV in the bacterial biomass (90/10), suggesting that both monomers had the same reactivity toward decarboxylation and alkene production. The gas composition roughly approached that produced by HT of pure PHB, with an additional CO_2 production (probably due to decarboxylation that involved non-PHA biomass) and only

DOI: 10.1021/acs.est.7b03927
Environ. Sci. Technol. 2017, 51, 12683−12691

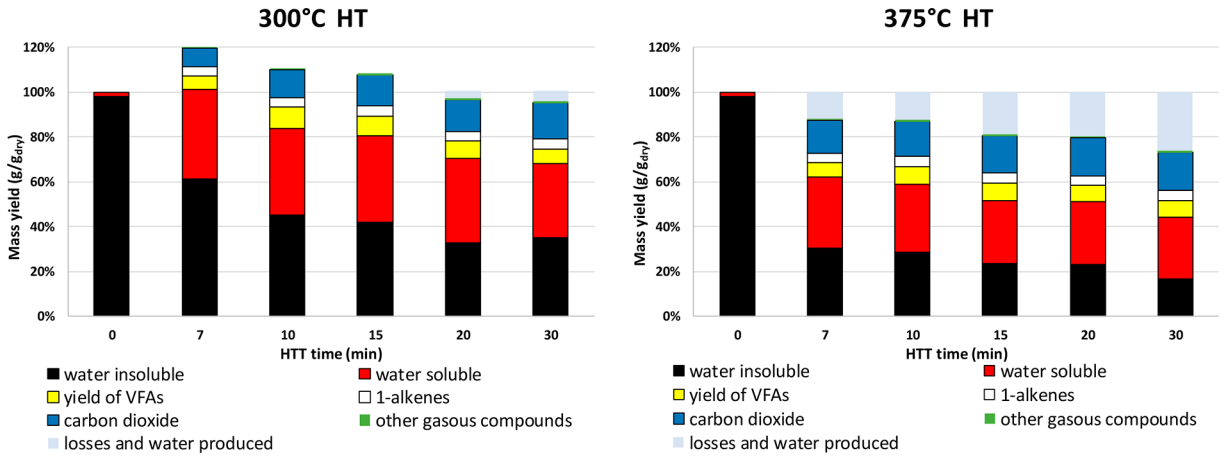


Figure 3. mass yield (on dry basis) of fractions produced by HT of 10% w_{dry}/w_{slurry} of MMC biomass with 13% w/w_{dry} content of PHA.

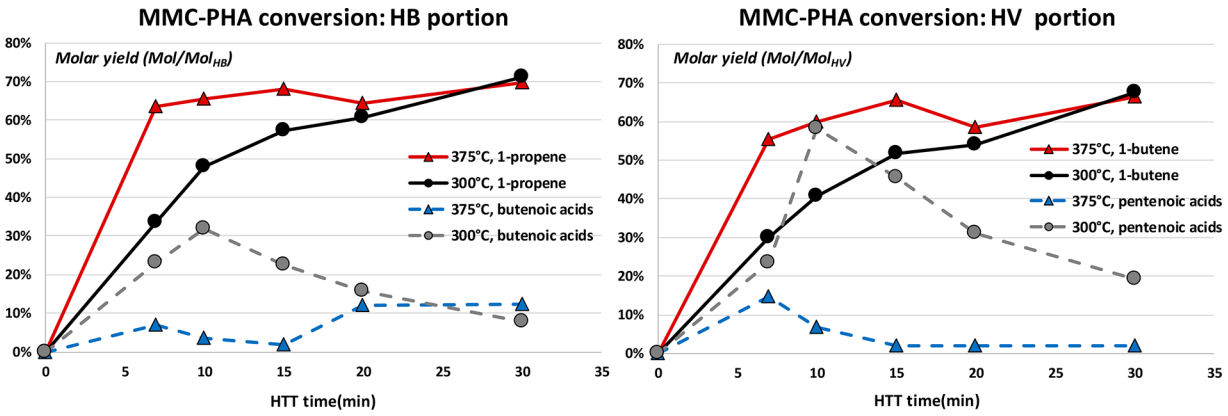


Figure 4. molar yield of intermediates produced by HT of 10% w_{dry}/w_{slurry} of MMC biomass with 13% w/w_{dry} content of PHA.

Table 2. Proximate Analysis of Water Insoluble Material Produced by HT of 10% w_{dry}/w_{slurry} of MMC Biomass with 13% w/w_{dry} Content of PHA

temperature		375	375	375	375	375	300	300	300	300	300
time (min)		7	10	15	20	30	7	10	15	20	30
wet solid DM[a]	20	19	32	30	46	68	20	22	24	26	31
C % w/w	36	20	22	27	30	37	39	40	31	46	39
N % w/w	5	2	2	2	2	3	3	3	3	4	3
H % w/w	5	4	3	3	3	5	5	4	4	6	4
S % w/w	1	1	2	2	3	1	0	1	1	1	1
O % w/w	40	31	20	18	11	27	36	29	31	6	14
ash % w/w	12	43	53	48	52	26	17	23	25	39	39

[a]Dry matter in vacuum filtration cake.

minor amount of CO and H_2. At both 300 and 375 °C, the highest gas yield was obtained after 30 min (Figure 3).

The evaluation of PHA degradation intermediates provided information about the kinetics of PHA depolymerization and decarboxylation, suggesting a clear effect of reaction conditions on yields and solubilization of MMC biomass (Figure 4). Supercritical HT at 375 °C produced a sharper increase in alkenes production, as the reaction seemed mostly completed within the first 5 min. A minimal production of depolymerization intermediates (alkenoic acids) was observed and an almost stable alkenes yield was obtained after 5−10 min. Subcritical HT at 300 °C produced a more gradual increase in the yield of alkenes until 30 min, with a significant production of alkenoic acids even after 30 min of reactions. Maximum alkenes yield was 64% and 71% of theoretical yields obtainable from a complete depolymerization or decarboxylation of PHA into alkenes and CO_2, respectively. After 30 min, HT yielded a residual amount of alkenoic acids (mostly trans-2-alkenoic acid) equal to 8−12% of the theoretical yield. Similar trends were observed for both hydroxybutyrate (Figure 4a) and hydroxyvalerate (Figure 4b) monomers, with a significant higher yield of depolymerization intermediates for HV at 300 °C.

HT at subcritical conditions (300 °C) applied for less than 10 min converted most of the non-PHA biomass into aqueous soluble products, leaving a residue mainly constituted by partially unreacted solids. Sum of the yield of all products approached 100%, suggesting negligible losses or water production. When HT was applied for short times, an apparent

DOI: 10.1021/acs.est.7b03927
Environ. Sci. Technol. 2017, 51, 12683−12691

Table 3. Results from Chemical Characterization of Aqueous Fraction by HT of 10% w_{dry}/w_{slurry} of MMC Biomass with 13% w/w_{dry} Content of PHA[a]

temperature	375	375	375	375	375	300	300	300	300	300
time (min)	7	10	15	20	30	7	10	15	20	30
OM[b]	40	41	38	38	37	46	49	51	43	41
TOC[c]	19	15	15	14	12	17	17	16	22	19
TN[d]	2.8	2.8	2.9	2.5	3.6	1.3	2.7	2.9	2.4	2.6
TAN[e]	1.1	1.1	1.3	1.2	1.3	0.7	1.1	1.2	1.0	1.0
VFA	5.1	7.0	5.3	5.7	5.3	2.1	4.3	5.1	5.1	5.0
PHA derivatives	1.1	0.5	0.3	1.5	1.6	3.3	4.9	3.5	2.4	1.3
hydroxyacids	4.4	5.3	3.3	2.0	1.6	5.9	7.0	1.4	2.9	2.8
cyclic dipeptides	0.8	1.1	1.2	0.6	0.7	0.2	1.8	1.4	1.2	1.9
glycerol	6.0	8.5	5.2	3.6	3.6	0.9	1.2	1.5	1.9	1.4
pyroglutamic acid	12	7.1	0.9	0.3	0.1	1.9	2.1	1.6	1.6	2.2
decarboxylated amino acids	0.7	0.6	0.7	0.5	0.5	0.0	0.0	0.0	0.1	0.1
ethanediol	1.3	1.3	0.1	0.0	0.0	0.9	1.0	1.0	0.9	0.8
nitrogenated heterocycles	0.9	1.5	1.3	1.0	1.1	0.1	0.1	0.1	0.1	0.1
others soluble substances	6	5	17	20	20	31	26	36	30	24

[a]All data are expressed in g L^{-1}. [b]Organic matter content. [c]Total organic carbon. [d]Total nitrogen. [e]Total ammonia nitrogen.

slight increase in total organic mass in the system was determined, which could be explained by a partial hydrolysis of the starting material and production of hydrated soluble products (e.g., glucose from carbohydrates or amino acids from peptides) that ended up in the HTW. At longer time, the amount of HTW organics remained almost stable (40−33% mass yield). During this last stage, an additional gas production, accompanied by an equivalent decrease in solid yield (final solid yield of 43% w/w), was observed.

On the opposite, HT at supercritical conditions (375 °C) produced a balanced distribution of gas and HTW constituents, and the water insoluble residue yield dropped immediately (within the first 7 min) to 32−28% mass yield. This effect was also demonstrated by the physical properties of the obtained solid residue, a relatively hydrophobic material with a sticky filtration residue with more than 68% dry weight (Table 2). The filtration residue produced at 300 °C appeared as a brown powder, with 31% dry weight, just slightly higher than that obtainable from raw biomass filtration (20% dry weight). This change in filtration behavior could be attributed to the decomposition of extracellular polymers and is well-known in the literature concerning heat treatment of sludge.[39,40]

Chemical Composition of HTW and Its Acidogenic Fermentation. HT of PHA-containing biomass converted roughly half of the dry matter into aqueous soluble substances, namely VFA, alkenoic acids from PHA degradation, other water-soluble substances from non-PHA biomass (HT products from degradation of proteins, carbohydrates and lipids) and products from cross-reactions during HT (Table 3). The water-soluble fraction included many constituents with different biodegradability and toxicity toward metabolic activities of anaerobic microbial consortia. Some compounds (e.g., sugars, glycerol, carboxylic acids, amino acids) can be considered as nontoxic metabolites, and they can be potentially used as carbon source to produce microbial biomass and short chain VFA. Other compounds like nitrogen-containing molecules (e.g., pyroglutamic acid, cyclic dipeptides, and amines) are known to be biodegradable but they usually have a degree of toxicity on specific microorganisms.[41,42] Finally, certain compounds can definitely inhibit the biological activity, also in traces amount (e.g., hydroxypyridines).[43] The most abundant compounds detected under all tested conditions were glycerol (up to 8.5 g L^{-1} in 375 °C for 10 min) and pyroglutamic acid (up to 7 g L^{-1}), with a relevant amount of hydroxyacids (up to 7 g L^{-1}, mainly C5 and C6 hydroxyacids). Noticeably, a significant content (up to 1.1 g L^{-1}) of nitrogen heterocycles (e.g., hydroxypyridines) was found in the HTW obtained at 375 °C. According to the yield of alkenes and water-soluble amount/composition, HTW from test performed at 300 °C for 30 min and 375 °C for 30 min were selected for batch and semicontinuous acidogenic fermentation tests (Figure 5).

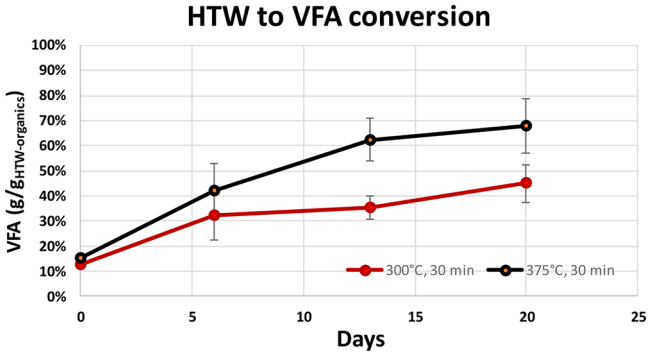


Figure 5. Acidogenic fermentation of aqueous product over time (20 days), expressed as $g_{VFA}/g_{HTW\text{-}organics}$ (%). Error bar shows the standard deviation (n = 2).

In batch acidogenic tests, VFA content increased from 13 to 15% of the HTW organics (before fermentation) to 45% and 68% for HTW obtained at 300 °C for 30 min and 375 °C for 30 min, respectively. This showed that, without considering toxicity (intrinsically relieved by the dilution of the test), the acidogenic inoculum was effectively able to metabolize a large portion of organics in HTW, producing VFA with relatively high yield.

To reveal the toxicity issue, a semicontinuous experiment was performed by a daily addition of undiluted HTW to syringe reactor previously fed with glucose (Figure 6). From a quantitative point of view, continuous fermentation of HTW with a HRT of 10 days converted a significant portion of the organic matter into VFA, whose concentration gradually

DOI: 10.1021/acs.est.7b03927
Environ. Sci. Technol. 2017, 51, 12683−12691

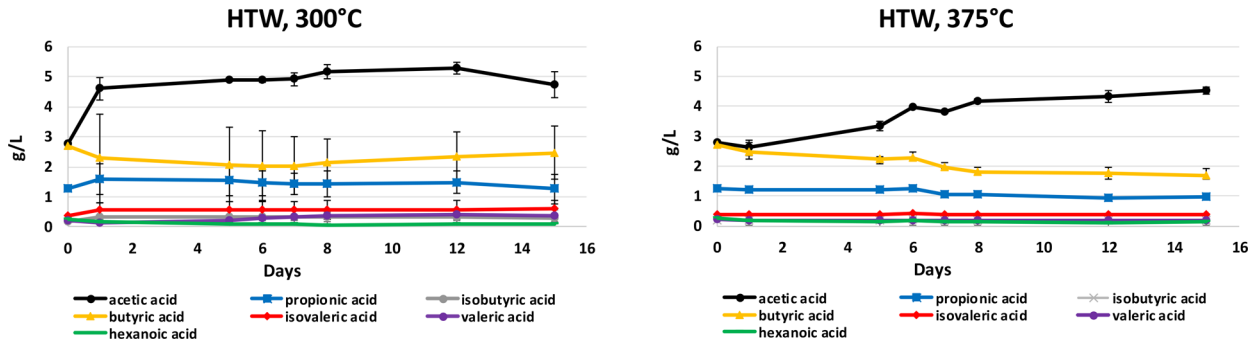


Figure 6. 15-days trend of VFA concentration (g L^{-1}) that followed the switch from glucose solution to HTW at 2 mL day^{-1}.

increased to a stable value between 8 ± 0.1 g L^{-1} for HTW obtained at 375 °C and 10 ± 2 g L^{-1} for HTW obtained at 300 °C. The concentration of other abundant GC-MS detectable compounds in HTW drastically decreased due to fermentation (Table 4). The main organic constituents of HTW (glycerol and pyroglutamic acid) were not even detectable in the digester outlet, whereas ethanediol, amines (decarboxlated aminocids) and aromatic heterocycles (e.g., hydroxypyridines), present in a significant amount in 375 °C HTW, were more persistent, though not completely degraded under the conditions of this study (see Table 4). This could be due to an incomplete degradation or, for certain compounds (e.g., decarboxylated amino acids and ethanediol) to an additional biological formation during the fermentation of HTW. In fact, the GC-MS of raw HTW (Figure 7) showed a broad hump of unresolved compounds between 15 and 30 min, attributable to many minor constituents that can be formed during HT of complex material like PHA-containing microbial biomass. These compounds probably represent a relevant fraction of the HTW, but they cannot be identified with the analytical procedures here used. Nonetheless, GC−MS analysis showed the disappearance of the 15−30 hump in the outlet, suggesting a removal of these constituents from the aqueous solution by degradation and/or precipitation during fermentation. This fact confirms the general feasibility of HTW recycle as shown in Figure 1. The final solution obtained under steady state (last 10 days of fermentation experiment) showed a VFA distribution like the one used for feeding bacteria and producing PHA-enriched MMC, but with a three times higher overall concentration. This clearly suggests the potential feasibility of the concept shown in Figure 1, including the recycling (with an eventual adjustment of C/N/P ratio) of fermented HTW for MMC production.

Table 4. % Relative Decrease of Concentration of Main Classes of Compound in Continuous Acidogenic Fermentation of HTW

	375 °C HTW % *decrease*	300 °C HTW % *decrease*
PHA derivatives	100	100
hydroxyacids	97.9	99.4
cyclic dipeptides	99.4	99.1
glycerol	100	100
pyroglutamic acid	99.8	100
decarboxylated amino acids	96.9	93.0
ethanediol	93.4	99.9
nitrogen heterocycles	98.5	98.4

In conclusion, hydrothermal treatment at subcritical (300 °C) and supercritical (375 °C) conditions was successfully applied to PHA-containing microbial biomass (PHA content of 13% w/w) to produce a high yield of alkenes with high selectivity. Through this approach, non-PHA biomass was

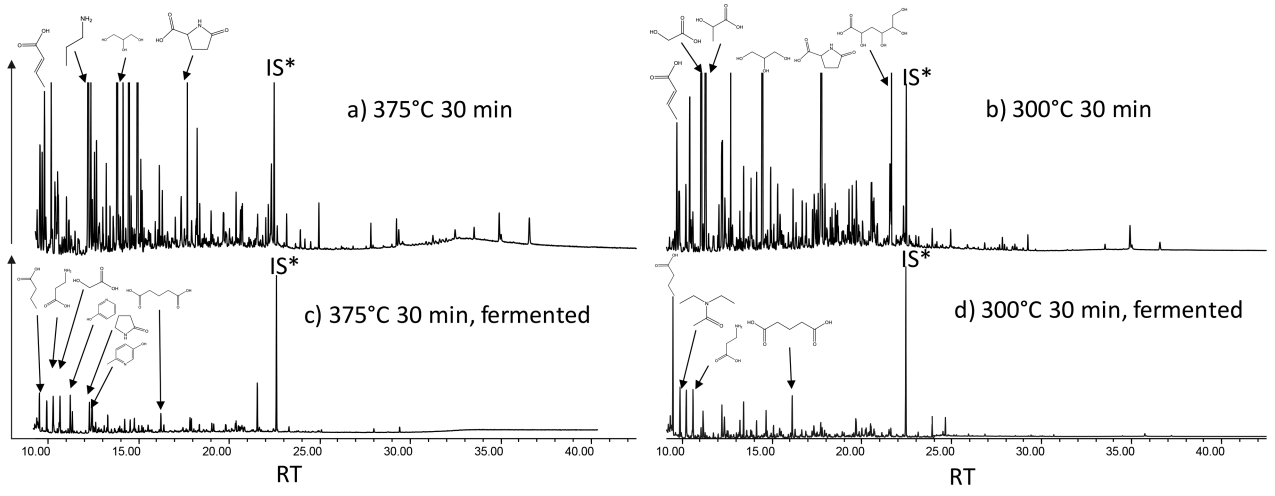


Figure 7. Comparison of GC-MS analysis of HTW obtained at 375 °C for 30 min (a) and 300 °C for 30 min (b) and corresponding fermented HTW (c,d). HTW and fermented HTW were dried at 60 °C and silylated, all compounds were identified as trimethylsilyl derivatives. *internal standard (sorbitol).

DOI: 10.1021/acs.est.7b03927
Environ. Sci. Technol. 2017, 51, 12683−12691

converted into a water insoluble residue and an aqueous solution (HTW) that included more than 33% of the whole organic matter. Organics in the HTW were proved to be acidogenically fermentable to produce VFA, ideal substrates for an additional PHA production by MMC. Insoluble solids retained most of the ashes and 37% of the original carbon; moreover, these materials were more hydrophobic and easier to dewater than the original biomass and, upon dewatering, could follow the same valorization strategies used for raw sludge (e.g., energy recovery). According to the results here presented, HT of MMC with moderate PHA content can produce about 50 kg of alkenes per tonne of suspended solids treated. In addition, HT implies a 60−70% decrease of sludge mass (hypothesizing simple solid/liquid separation) and 80% decrease of wet sludge volume.

AUTHOR INFORMATION

Corresponding Authors

*(C.T.) Phone: 0039-0544937351; e-mail: cristian.torri@unibo.it.

*(D.W.F.B.) E-mail: d.w.f.brilman@utwente.nl.

ORCID

Cristian Torri: 0000-0001-5233-6786

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Peccia, J.; Westerhoff, P. We Should Expect More out of Our Sewage Sludge. *Environ. Sci. Technol.* **2015**, *49* (14), 8271−8276.

(2) Carrère, H.; Dumas, C.; Battimelli, A.; Batstone, D. J.; Delgenès, J. P.; Steyer, J. P.; Ferrer, I. Pretreatment methods to improve sludge anaerobic degradability: A review. *J. Hazard. Mater.* **2010**, *183* (1), 1−15.

(3) Kelessidis, A.; Stasinakis, A. S. Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. *Waste Manage.* **2012**, *32* (6), 1186−1195.

(4) Kacprzak, M.; Neczaj, E.; Fijałkowski, K.; Grobelak, A.; Grosser, A.; Worwag, M.; Rorat, A.; Brattebo, H.; Almås, Å.; Singh, B. R. Sewage sludge disposal strategies for sustainable development. *Environ. Res.* **2017**, *156*, 39−46.

(5) Gude, V. G. Energy positive wastewater treatment and sludge management. *Edorium J. Waste Manage* **2015**, *1*, 10−15.

(6) Van Loosdrecht, M. C. M. Importance of bacterial storage polymers in bioprocesses. *Water Sci. Technol.* **1997**, *35* (1), 41−47.

(7) Oliveira, C. S. S.; Silva, C. E.; Carvalho, G.; Reis, M. A. Strategies for efficiently selecting PHA producing mixed microbial cultures using complex feedstocks: Feast and famine regime and uncoupled carbon and nitrogen availabilities. *New Biotechnol.* **2017**, *37*, 69−79.

(8) Serafim, L. S.; Lemos, P. C.; Albuquerque, M. G. E.; Reis, M. A. M. Strategies for PHA production by mixed cultures and renewable waste materials. *Appl. Microbiol. Biotechnol.* **2008**, *81* (4), 615−628.

(9) Reis, M. A. M.; Serafim, L. S.; Lemos, P. C.; Ramos, A. M.; Aguiar, F. R.; Van Loosdrecht, M. C. M. Production of polyhydroxyalkanoates by mixed microbial cultures. *Bioprocess Biosyst. Eng.* **2003**, *25* (6), 377−385.

(10) Takabatake, H.; Satoh, H.; Mino, T.; Matsuo, T. PHA (polyhydroxyalkanoate) production potential of activated sludge treating wastewater. *Water Sci. Technol.* **2002**, *45* (12), 119−126.

(11) Yan, S.; Tyagi, R. D.; Surampalli, R. Y. Polyhydroxyalkanoates (PHA) production using wastewater as carbon source and activated sludge as microorganisms. *Water Sci. Technol.* **2006**, *53* (6), 175−180.

(12) Valentino, F.; Morgan-Sagastume, F.; Campanari, S.; Villano, M.; Werker, A.; Majone, M. Carbon recovery from wastewater through bioconversion into biodegradable polymers. *New Biotechnol.* **2017**, *37*, 9−23.

(13) Morgan-Sagastume, F.; Hjort, M.; Cirne, D.; Gérardin, F.; Lacroix, S.; Gaval, G.; Karabegovic, L.; Alexandersson, T.; Johansson, P.; Karlsson, A.; Bengtsson, S.; Arcos-Hernández, M. V.; Magnusson, P.; Werker, A. Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale. *Bioresour. Technol.* **2015**, *181*, 78−89.

(14) Villano, M.; Valentino, F.; Barbetta, A.; Martino, L.; Scandola, M.; Majone, M. Polyhydroxyalkanoates production with mixed microbial cultures: from culture selection to polymer recovery in a high-rate continuous process. *New Biotechnol.* **2014**, *31* (4), 289−296.

(15) Gao, X.; Chen, J. C.; Wu, Q.; Chen, G. Q. Polyhydroxyalkanoates as a source of chemicals, polymers, and biofuels. *Curr. Opin. Biotechnol.* **2011**, *22* (6), 768−774.

(16) Samori, C.; Abbondanzi, F.; Galletti, P.; Giorgini, L.; Mazzocchetti, L.; Torri, C.; Tagliavini, E. Extraction of polyhydroxyalkanoates from mixed microbial cultures: impact on polymer quality and recovery. *Bioresour. Technol.* **2015**, *189*, 195−202.

(17) Ren, Q.; Ruth, K.; Thöny-Meyer, L.; Zinn, M. Enatiomerically pure hydroxycarboxylic acids: current approaches and future perspectives. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 41−52.

(18) Zhang, X.; Luo, R.; Wang, Z.; Deng, Y.; Chen, G. Q. *Biomacromolecules* **2009**, *10* (4), 707−711.

(19) Torri, C.; Cordiani, H.; Samorì, C.; Favaro, L.; Fabbri, D. Fast procedure for the analysis of poly(hydroxyalkanoates) in bacterial cells by off-line pyrolysis/gas-chromatography with flame ionization detector. *Journal of Chromatography A* **2014**, *1359*, 230−236.

(20) Ariffin, H.; Nishida, H.; Hassan, M. A.; Shirai, Y. Chemical recycling of polyhydroxyalkanoates as a method towards sustainable development. *Biotechnol. J.* **2010**, *5*, 484−492.

(21) Wagner, J.; Bransgrove, R.; A. Beacham, T.; J. Allen, M. J.; Meixner, K.; Drosg, B.; Ting, V. P.; Chuck, C. J. Co-production of bio-oil and propylene through the hydrothermal liquefaction of polyhydroxybutyrate producing cyanobacteria. *Bioresour. Technol.* **2016**, *207*, 166−174.

(22) Itoh, S.; Suzuki, A.; Nakamura, T.; Yokoyama, S.-Y. Production of heavy oil from sewage sludge by direct thermochemical liquefaction. *Desalination* **1994**, *98* (1), 127−133.

(23) Qian, L.; Wang, S.; Savage, P. E. Hydrothermal liquefaction of sewage sludge under isothermal and fast conditions. *Bioresour. Technol.* **2017**, *232*, 27−34.

(24) Zhai, Y.; Chen, H.; Xu, B.; Xiang, B.; Chen, Z.; Li, C.; Zeng, G. Influence of sewage sludge-based activated carbon and temperature on the liquefaction of sewage sludge: yield and composition of bio-oil, immobilization and risk assessment of heavy metals. *Bioresour. Technol.* **2014**, *159*, 72−79.

(25) Vardon, D. R.; Sharma, B. K.; Scott, J.; Yu, G.; Wang, Z. C.; Schideman, L.; Zhang, Y. H.; Strathmann, T. J. Chemical properties of biocrude oil from the hydrothermal liquefaction of *spirulina* algae, swine manure, and digested anaerobic sludge. *Bioresour. Technol.* **2011**, *102* (17), 8295−8303.

(26) Wang, Y.; Chen, G.; Li, Y.; Yan, B.; Pan, D. Experimental study of the bio-oil production from sewage sludge by supercritical conversion process. *Waste Manage.* **2013**, *33* (11), 2408−2415.

(27) Suzuki, A.; Nakamura, T.; Yokoyama, S. Y. Effect of operating parameters on thermochemical liquefaction of sewage sludge. *J. Chem. Eng. Jpn.* **1990**, *23* (1), 6−11.

(28) Yokoyama, S. Y.; Suzuki, A.; Murakami, M.; Ogi, T.; Koguchi, K.; Nakamura, E. Liquid fuel production from sewage sludge by catalytic conversion using sodium carbonate. *Fuel* **1987**, *66* (8), 1150−1155.

(29) Bougrier, C.; Delgenès, J. P.; Carrère, H. Effects of thermal treatments on five different waste activated sludge samples solubilisation, physical properties and anaerobic digestion. *Chem. Eng. J.* **2008**, *139* (2), 236−244.

(30) Carrère, H.; Dumas, C.; Battimelli, A.; Batstone, D. J.; Delgenès, J. P.; Steyer, J. P.; Ferrer, I. Pretreatment methods to improve sludge anaerobic degradability: A review. *J. Hazard. Mater.* **2010**, *183* (1), 1−15.

DOI: 10.1021/acs.est.7b03927
Environ. Sci. Technol. 2017, 51, 12683−12691

(31) Morgan-Sagastume, F.; Pratt, S.; Karlsson, A.; Cirne, D.; Lant, P.; Werker, A. Production of volatile fatty acids by fermentation of waste activated sludge pre-treated in full-scale thermal hydrolysis plants. *Bioresour. Technol.* **2011**, *102* (3), 3089−3097.
(32) Posmanik, R.; Labatut, R. A.; Kim, A. H.; Usack, J. G.; Tester, J. W.; Angenent, L. T. Coupling hydrothermal liquefaction and anaerobic digestion for energy valorization from model biomass feedstocks. *Bioresour. Technol.* **2017**, *233*, 134−143.
(33) Stuckey, D. C.; McCarty, P. L. The effect of thermal pretreatment on the anaerobic biodegradability and toxicity of waste activated sludge. *Water Res.* **1984**, *18* (11), 1343−1353.
(34) Tommaso, G.; Chen, W. T.; Li, P.; Schideman, L.; Zhang, Y. Chemical characterization and anaerobic biodegradability of hydrothermal liquefaction aqueous products from mixed-culture wastewater algae. *Bioresour. Technol.* **2015**, *178*, 139−146.
(35) Si, B. C.; Li, J. M.; Zhu, Z. B.; Zhang, Y. H.; Lu, J. W.; Shen, R. X.; Zhang, C.; Xing, X. H.; Liu, Z. Continuous production of biohythane from hydrothermal liquefied cornstalk biomass via two-stage high-rate anaerobic reactors. *Biotechnol. Biofuels* **2016**, *9*, 254.
(36) Torri, C.; Fabbri, D. Biochar enables anaerobic digestion of aqueous phase from intermediate pyrolysis of biomass. *Bioresour. Technol.* **2014**, *172*, 335−341.
(37) Oasmaa, A.; Kuoppala, E. Solvent Fractionation Method with Brix for Rapid Characterization of Wood Fast Pyrolysis Liquids. *Energy Fuels* **2008**, *22* (6), 4245−4248.
(38) Alba, L. G.; Torri, C.; Fabbri, D.; Kersten, S. R. A.; Brilman, D. W. F. Microalgae growth on the aqueous phase from Hydrothermal Liquefaction of the same microalgae. *Chem. Eng. J.* **2013**, *228*, 214−223.
(39) Mikkelsen, L. H.; Keiding, K. Physico-chemical characteristics of full scale sewage sludges with implications to dewatering. *Water Res.* **2002**, *36* (10), 2451−2462.
(40) Neyens, E.; Baeyens, J. A review of thermal sludge pre-treatment processes to improve dewaterability. *J. Hazard. Mater.* **2003**, *98* (1), 51−67.
(41) Cho, J. Y.; Choi, J. S.; Kang, S. E.; Kim, J. K.; Shin, H. W.; Hong, Y. K. Isolation of antifouling active pyroglutamic acid, triethyl citrate and di-n-octylphthalate from the brown seaweed Ishige okamurae. *J. Appl. Phycol.* **2005**, *17*, 431−435.
(42) Borthwick, A. D. 2,5-Diketopiperazines: Synthesis, Reactions, Medicinal Chemistry, and Bioactive Natural Products. *Chem. Rev.* **2012**, *112* (7), 3641−3716.
(43) Liu, S. M.; Wu, C. H.; Huang, H. J. Toxicity and anaerobic biodegradability of pyridine and its derivatives under sulfidogenic conditions. *Chemosphere* **1998**, *36* (10), 2345−2357.

DOI: 10.1021/acs.est.7b03927
Environ. Sci. Technol. 2017, 51, 12683−12691