Biological treatment of Hydrothermal Liquefaction (HTL) wastewater: Analytical evaluation of continuous process streams

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[**] *This paper is dedicated to the memory of Eng. Roberta Miglio who passed away on January 2020*

Abstract

In order to deal with hydrothermal liquefaction wastewater (HTWW), a new anaerobic-aerobic continuous process was developed. The process, which included a sequence of Up-Flow Anaerobic Sludge Blanket (UASB) and downstream aerobic Continuously Stirred Tank Reactor (CSTR), was tested on the HTWW obtained from Waste to Fuel® demo plant developed by ENI s.p.a. [1]. Performance of the system was evaluated in term of methane yield and chemical oxygen demand (COD) abatement capability. Detailed fate of organic compounds was evaluated through different analytical techniques, highlighting main issues and potential of HTWW biological treatment. The system was fed with neat HTWW (189 gCOD L^{-1}) for 2.5 y, with variable organic loading rate (OLR) and minimal external inputs. UASB reactors converted most of HTWW organics into volatile fatty acids (VFA) and methane with concurrent precipitation of oily like insoluble, whereas aerobic CSTR removed VFA from anaerobic effluent. Under regime conditions (ORL equal to 0.5 gCOD L^{-1} d^{-1}) COD decreased from 189 to 6.6 gCOD L^{-1}, showing 97% COD abatement with the coupled anaerobic-aerobic treatment. Such a COD abatement was obtained by means of multiple effects, namely biomethanation, precipitation of organic matter and aerobic oxidation of fermentation products (VFA produced in anaerobic digestion) and subsequent aerobic oxidation (in downstream aerobic reactor). These effects accounted for 43, 40 and 17% of the total COD decrease, respectively. Inhibition phenomena were the key challenge for improving methane yields and system productivities. The overall results confirmed that valorization of HTWW is a feasible task, albeit rather challenging.

1. Introduction

Food Waste and, more in general, wet biomass resources are considered to be a pivotal in providing renewable and carbon neutral alternatives to fossil fuels. Hydrothermal liquefaction (HTL), being operated at moderate temperatures (250-375°C) and residence times between 15-120 min, represents a versatile thermochemical method for conversion of biomass and waste into a biocrude, solid residue and aqueous byproduct [2,3]. This technology has received significant attention in the last decades and recently outcropped into process demonstration [4] and early commercialization of interesting applications. In this context, ENI s.p.a. recently scaled-up a complete Waste to Fuel® process able to deal with sorted organic fraction of municipal solid waste (OMSW), which allows the production and co-processing (e.g. ENI slurry technology®) of biocrude in existing refineries. Byproduct valorization or disposal strategy still represents an important issue worth of investigation. Whereas solid residue (somewhat called hydrochar) is characterized by minor mass yield and can be used as solid fuel, aqueous byproduct (HTWW) is the most relevant product in terms of mass yield, and usually retain 30-40% of chemical energy of the feedstock [5]. Due to its chemical properties, namely high Chemical Oxygen Demand (COD) and inherent toxicity, it cannot be discharged in wastewater treatment systems and requires reliable recovery strategy [6]. HTWW organics are a complex mixture of organics, whose concentration, investigated by several authors, is a function of feedstock composition and process conditions [7–11]. In order to valorize soluble organics and to decrease COD to acceptable levels, biological treatments, such as anaerobic digestion (AD) and related process, have been proposed [12] and tested by several authors [13–27]. Optimal biocrude yields are typically obtained using with 280-375°C temperature range and 30-60 min residence time [26,28,29]. However, at these conditions HTWW are generated with significant levels of non-biodegradable organics and high relative toxicity [30,31] that hamper biological conversion pathways. Several authors investigated biological processing of HTWW obtained with different feedstock or HTL parameters. Most studies tested AD of HTWW, providing a preliminary insight about key concerns of anaerobic biological processing. To the end of addressing toxicity issues, different approaches were adopted, among which simple dilution and pretreatment of HTWW. Most of the above mentioned studies were concerned with batch tests conducted on diluted HTWW. At best of our knowledge, there is a paucity of studies on continuous biological processing of this type of effluents [32]., The present work aimed at investigating a continuous biological treatment of HTWW in order to evaluate positive aspects and critical points. The treatment of HTWW consisted in anaerobic-aerobic multistage process, based on ENI-INSTM patent [33], conducted for an overall timespan of three years. In particular, a detailed monitoring of the system was performed for six months under steady conditions. As a term of comparison, all data were expressed using COD as a unit of measured quantity. Besides being a parameter that defines wastewater quality, COD (or theoretical oxygen demand) is a direct measurement of chemical energy. Both COD (from stoichiometry) and higher heating value (HHV) (empirically) are linearly correlated to elemental compositions [34]. Hence, 1 kg of natural

68 occurring COD typically contains 15 MJ of chemical energy and can be transformed into heat, work (with a
69 certain efficiency) or, through anaerobic process into maximum 1 kg of COD of chemicals or materials. The
70 COD of common feedstock and fermentation products range between roughly 1 kgO_2/kg (carbohydrates)
71 and 4 kgO_2/kg (methane). Therefore, establishing COD balance and conversion rate allows to compare
72 largely different conditions. Detailed fate of organic compounds was established performing a COD balance
73 among different compounds and compounds groups, highlighting main issues and potential of HTWW
74 biological treatment.
75

76 2. Materials and methods

77 2.1 HTL wastewater obtainment

78 The Waste to Fuel® pilot plant has a bio-oil production capacity of approximately 70 kg per day and is
79 supplied with a maximum of 700 kg of sorted organic waste per day. The plant is a continuous one able to
80 work 24 h a day and it is composed by two main sections, the first one named as homogenization and the
81 second one liquefaction section. In the first section, the sorted organic fraction of municipal solid waste
82 (OMSW) is introduced as such, without any kind of pretreatment. The goal of this first section is only to
83 homogenize the waste in order to achieve a constant chemical composition in the continuous feed. In the
84 following liquefaction section, the hydrothermal liquefaction transformation was performed introducing
85 hot homogenized stream into a plug flow reactor. Average operative conditions are the following: 260-300
86 °C, pressure up to 90 bar with a residence time of about two hours [35]. After reaction, the hot product
87 stream is sent to a separation step in order to recover bio-oil and the other byproducts, such as gas phase,
88 a solid fraction and water phase (thereafter named HTWW).

89 HTWW was cooled down and stored at ambient temperature. Different samples of about 30 kg each were
90 withdrawn and used in the present study. The OMSW and HTL products were routinely characterized in
91 order to control inlet quality and to calculate operative yields. An average composition of OMSW was the
92 following: water content 75-80 % w/w, inorganic content 10-22 % w/w (on dry basis), lipid content 10-15 %
93 w/w (on dry basis), protein 10-14 % (on dry basis). The elemental average analysis of the OMSW is reported
94 (on dry basis) as C 40 %. H 5.7 %, N 2.3 %, S 0.3 %.

95 2.2 Multistage Anaerobic-Aerobic Digestion

96 A biological multistage system was operating continuously for a period of 2.5 years in order to investigate
97 the biological potential of converting HTWW in biogas (anaerobic phase) and reducing COD (aerobic step)
98 in order to dispose HTWW into wastewater treatment plants. The process scheme was changed over time
99 for the first 230 days of operations ending up with the final configuration shown in Figure 1. IN is the
100 HTWW mixed with diluting stream (a portion of OUT) that enters the system, AA (after anaerobic digestion)

is the output of the anaerobic reactor and OUT is the final effluent after aerobic treatment used to dilute HTWW.

The definitive configuration consisted in a multistage digester (400 mL working volume) and an aerobic fermenter (200 mL working volume). The multistage anaerobic digester consisted in five sequential up-flow anaerobic sludge blanket (UASB) connected in series at thermophilic (UASB 1, 65°C) and mesophilic conditions (UASB 2 to UASB 5, 40°C). Initial thermophilic condition was chosen for process compatibility with the actual temperature of HTL outlet), and in order to speed up the hydrolysis phase. One of the basic requirements of this multistage system is that the system should perform a complete acidogenesis in the first thermophilic UASB. To guarantee this condition, product (VFA) inhibition should be avoided by means of dilution of the inlet streams to a COD level less than 20-30 g L^{-1} which is the maximum concentration of VFA that can be obtained under anaerobic condition at almost neutral pH. Therefore, in accordance with the tested chemical composition, HTWW was diluted through recirculation with the effluent from aerobic stage (1:10 ratio). In routine operations, about 20-25 mL d^{-1} of pre-diluted HTWW was pumped at the bottom of UASB 1 through a peristaltic pump. Since all UASB reactors are connected in series, about 20 mL/day (HRT 20 days) were collected with the resulting biogas in a laminated gas bag, placed at the end of the anaerobic system. The anaerobic effluent was then subjected to the aerobic fermenter (HRT 10 days). Finally, the aerobic effluent was used to dilute HTWW before entering the system. All UASB reactors were filled with a bacterial inoculum taken from an anaerobic digester treating stillage and wastewater sludge. Total suspended solids (TSS), volatile suspended solids (VSS) and COD were 56 g L^{-1}, 35 g L^{-1} 45 gO_2 L^{-1} respectively.

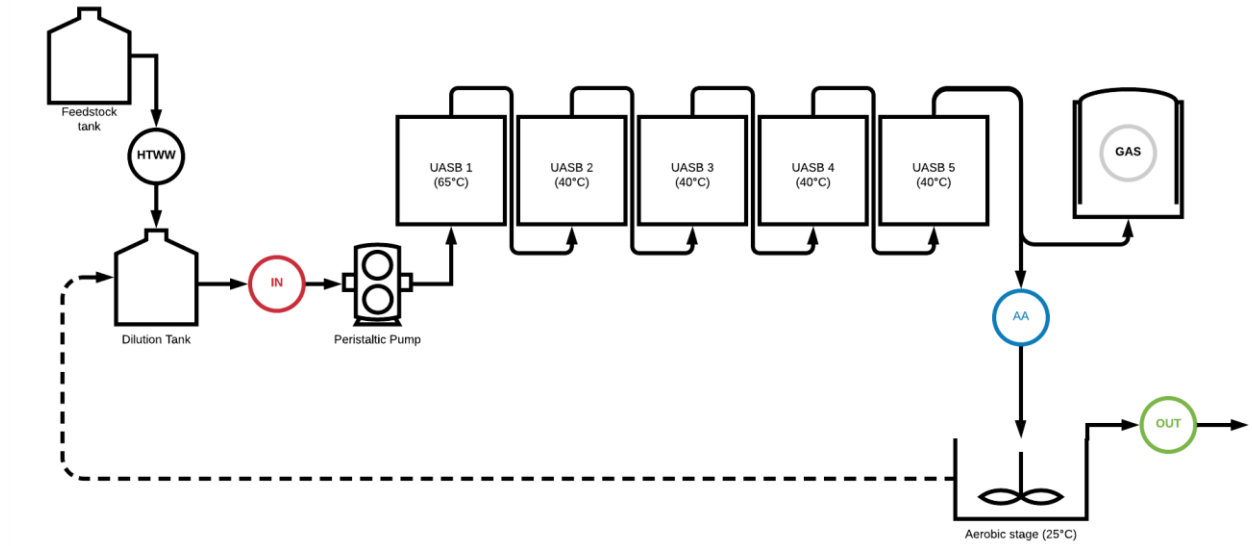

Figure 1: process scheme configuration of the Multistage Anaerobic-Aerobic Digestion

2.3 Chemical characterization scheme and definition of chemical classes.

The samples feedstock, HTWW and all aqueous streams (IN, OUT, AA see figure 1) obtained during experiments were characterized following the analytical scheme shown in figure 2. Samples typically consisted in a brownish aqueous slurry and all the analyses were performed within one day from the obtainment. The whole sample including suspended solids was diluted with deionized water and analyzed for COD in triplicate (total COD of the sample, tCOD). Thereafter the sample was centrifuged at 5000 rpm to obtain the soluble fraction, a clear dark-reddish supernatant solution, that was analysed for COD (soluble COD, sCOD). The amount of the insoluble COD (iCOD) was calculated by difference (iCOD= tCOD-sCOD). The insoluble fraction included suspended solids (e.g. bacterial biomass formed) and any organic precipitate (e.g. colloidal substances that precipitates upon HTWW aging). Samples of the soluble fraction were subjected to an array of analyses described in following sections. For the reasons highlighted in the introduction,

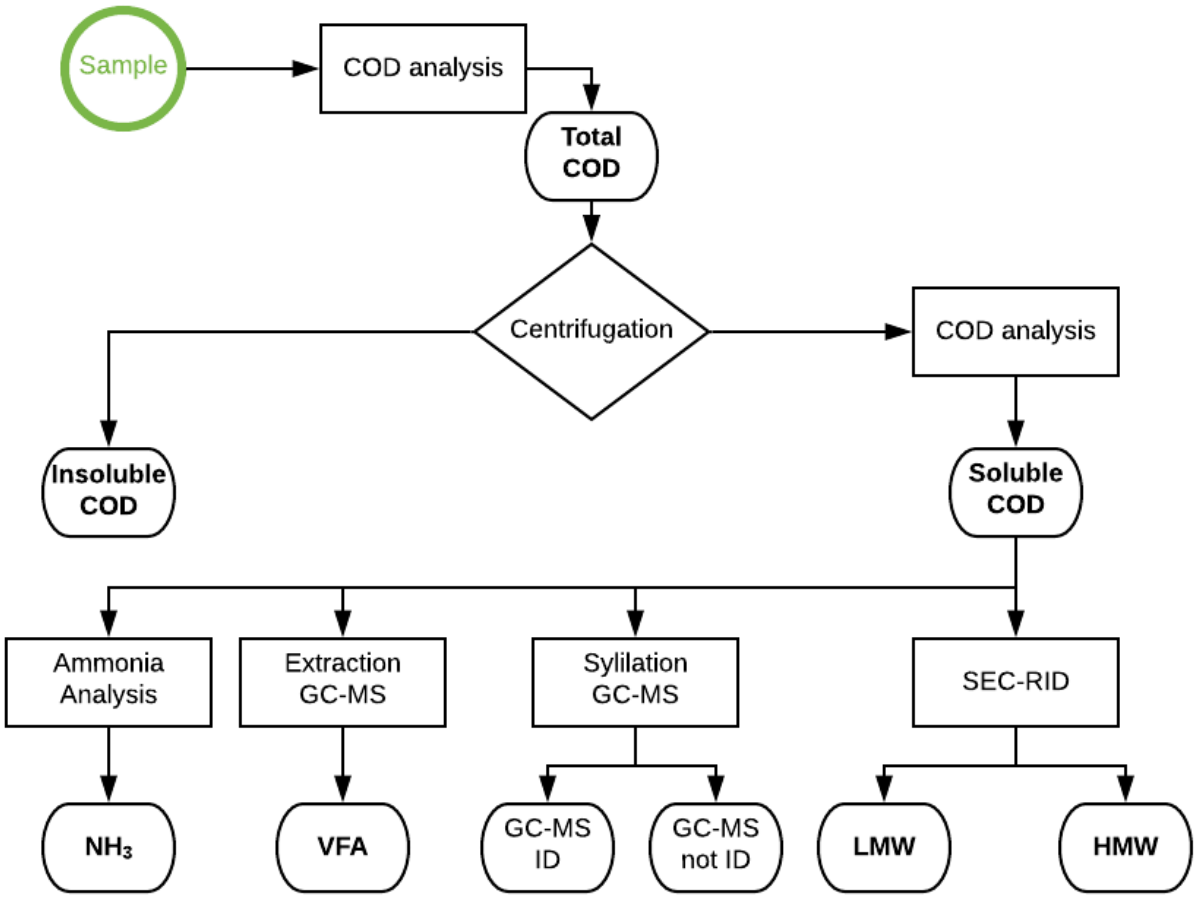

Figure 2: analytical scheme for the characterization of HTWW major components.

2.4 COD, Biogas, pH and total ammonia nitrogen (TAN)

COD was measured by thermal oxidation at 1200 °C with detection of the oxygen consumption using a COD analyzer QuickCODLab (LAR Process Analyzer AG) following the ASTM D6238-98 method. After proper dilution, the sample was injected directly into the reactor where it was completely oxidized at 1200 °C under air/nitrogen flow and continuously analyzed with an O_2 detector. The COD was calculated as g O_2 L^{-1} by comparison of signal areas (O_2 consumption) with those of known standard solution of glucose. All analysis was performed in triplicate.

Biogas production was measured volumetrically at 25 °C after withdrawal from the sampling bag and analyzed in terms of CH_4, CO_2, and H_2 by means of gas chromatography coupled with thermal conductivity detector (GC-TCD, Agilent 78120A) [36]. pH was determined with a pH meter after calibration with buffer solutions. Ammonia concentration was determined potentiometrically by means of an ammonia selective probe after calibration with standard solutions of ammonium chloride.

2.5 Quantitative analysis of GC-MS detectable compounds

VFA were analysed by solvent extraction and GC-MS according Ghidotti et al. [37]. An aliquot of HTWW before and after biological treatments was added with 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 solution was shaken and the upper phase, containing VFA dissolved in dimethyl carbonate, was analyzed by GC-MS. GC-MS analysis of VFA were performed using an Agilent 7820A gas chromatograph connected to an Agilent 5977E quadrupole mass spectrometer. The injection port temperature was 280 °C. Analytes were separated on a DB-FFAP polar column (30 m length, 0.25 μm i.d., 0.25 mm film thickness), with helium flow of 1 mL min^{-1}. Mass spectra were recorded under electron ionization (70 eV) at a frequency of 1 scan s^{-1} within the 29–450 *m/z* range. The temperature of the column was set to 50 °C (5 min) and increased to 250 °C (10 °C min^{-1}). 2-Ethylbutyrate (0.1 mL of a solution 1000 ppm) was used as internal standard for the quantitation; calibration was performed with commercial VFA solution.

The quantitation of GC-MS detectable organics in HTWW before and after biological treatments was performed after water evaporation and silylation as described in [38]. An aliquot of sample (0.1 mL) was spiked with 3-chlorobenzoic acid (50 μg) and dried under nitrogen flow. Subsequently, *bis*-trimethylsilyl-trifluoroacetamide with 1% trimethyltrichlorosilane (BSTFA+TMCS, 0.1 mL), acetonitrile (0.1 mL) and a drop of pyridine were added to the dried residue and heated at 60 °C for 2 h. The solution was finally spiked with recovery standard (methyl-nonandecanoate, 50 μg) diluted in ethyl acetate (0.5 mL) and analyzed by GC-MS. GC-MS analysis of silylated samples were performed using an Agilent HP 6850 gas chromatograph connected to an Agilent HP 5975 quadrupole mass spectrometer. The injection port temperature was 280

°C. Analytes were separated on a HP-5 fused-silica capillary column (stationary phase poly(5% diphenyl/95% dimethyl)siloxane, 30 m, 0.25 mm i.d., 0.25-µm film thickness), with helium as the 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 12–600 *m/z* range. The temperature of the column was increased from 50 to 180 °C at 50 °C min^{-1} and then from 180 to 300 °C at 5 °C min^{-1}. 3-chlorobenzoic acid (0.05 mL of a solution 1000 ppm) was used as internal standard for the quantitation, assuming a unitary response factor for all the detected analytes.

2.6 Size exclusion chromatography (SEC)

Molecular size distributions were determined by high performance liquid chromatography (HPLC) – size exclusion chromatography (SEC) analysis. The analyses were performed using a HPLC 1200-series system (Agilent Technologies, USA) equipped with a diode array detector (DAD, G1315D Agilent Technologies, USA) and a refractive index detector (RID, G1362A Agilent Technologies, USA). Separation was carried out at 35 °C and maximum of pressure of 400 bar on a PL aquagel-OH 20 column (300 x 7.5 mm, particle size 8 µm), obtained from Agilent Technologies. Ultrapure water was used as eluent at a flowrate of 1 mL min^{-1} and the injection volume was 20 µL. The parameter of DAD detector was a wavelength range from 190 to 400 nm. The RID detector was set at a temperature of 30 °C and operated under positive mode with zero offset of 5% and attenuation of 500.000 nRIU. A peak > 0.2 mm with response time 4 s and frequency of 2.31 Hz were used. The HTWW samples were filtered through PTFE syringe filters (0.45 µm) prior to analysis. The columns were equilibrated overnight before use. Data acquisition and analysis were performed with Agilent OpenLAB CDS Version 2.4.

2.7 Data presentation

Concentration and yields were expressed in term of COD. For every compound or group of compounds analyzed by means of GC-MS or silylation/GC-MS, COD concentrations ($gCOD_x\ L^{-1}$) was then calculated by multiplying the mass concentration ($g_x\ L^{-1}$) for the specific COD obtained from chemical formula ($gCOD\ g_x^{-1}$). To obtain the signal of unknown GC detectable constituents the area of identified compounds and blank was subtracted to the entire GC-MS area detected. This signal of unknown GC detectable compounds was then used to obtain the corresponding COD concentration ($gCOD_{GC\ unk}\ L^{-1}$) assuming a specific COD ($gCOD\ g_x^{-1}$) equal to the average value of identified compounds. COD concentration of non-detectable compounds (e.g. HMW) was calculated by subtraction of all COD concentration from tCOD of HTWW ($gCOD\ L^{-1}$).

3. Results and discussion

3.1 Chemical characterization of HTWW.

For the scope of this investigation, one HTWW batch was fully characterized and processed in a 2.5-year time span. This sample was obtained by HTL of the OMSW as described in section 2.1. The reddish-brown clear solution obtained by centrifugation is characterized by chemical oxygen demand equal to 189±10 gCOD L^{-1} and complex chemical composition, outlined in Table 1. The sample mostly consisted in water, concentration of non-volatile constituents (evaporation residue at 105°C) equal to 101±12 g/L, which includes 20±1 g L^{-1} of inorganics (residue at 600°C for 10 h). Inorganics composition is close to that expected from OMWS, with moderate salinity, presence of most micro and macro-nutrients needed for biomass growth and without significant amount of toxic metals. Total nitrogen (TN) concentration was 8.4 g L^{-1} of which 3.3 gN L^{-1} was the total ammonia nitrogen (TAN). These results are in line with the literature about hydrothermal liquefaction of food waste [8] with multicomponent characteristics [7]. Beyond standard characterization techniques, which includes COD, TN, TAN, VFA, metals, HTWW was subjected to additional complementary analyses. Main GC-MS detectable constituents included organic acids (principally VFA), short chain hydroxy acids (e.g. lactic and hydroxyacetic acid) and sugar derivatives (pentoses, anhydrohexoses and deoxy-gluconic acids). It is interesting to notice the presence of a relatively large amount of sorbitol and inositol, which are food specific organics that probable survived the HTL process and end up in the aqueous phase (e.g. fruit peel). Minor but relevant amount of low molecular weight N-containing aromatic heterocycles, here abbreviated as NAH (e.g. hydroxypyridines and pyrazines) were found. These NAH may represent a concern for the anaerobic/aerobic treatment of the solution due to their toxicity. From the quantitative point of view, HTWW composition was dominated by sugars and sugars derivatives, which accounted for 26% of the total COD. Other common biological intermediates, namely lactic acid, glycerol, VFA and long chain fatty acids accounted for 9%, 7%, 5% and 4% of COD respectively. Some specific HTL derivate organic compounds contributed significantly to the COD. For example, pyroglutamic acid and other hydroxy acids were found in 10 and 7.9 gCOD L^{-1} concentration, corresponding to 5 and 4% of the total COD. Finally, total concentration of toxicity concerning NAH was 9.9 gCOD L^{-1}, which means a non-negligible 5% contribution to total COD of the sample.

A large number of semivolatile compounds (about 10% of COD detected by GC could not be identified. Moreover, a significant (about 25%) fraction of COD was not detectable after silylation/GC-MS, being probably formed by extremely polar or high molecular weight organic compounds (HMW). To obtain additional information about this fraction, SEC-RID analysis of HTWW was performed (Figure 3), proving the molecular distribution of polar (compounds which affect the refraction index) dissolved organic matter. Blank subtracted SEC-RID graph (Figure 3) showed 4 broad peaks in the 0-1000 Da range. First two peaks, in the 60-90 Da and 180-300 Da ranges could be attributed to small polar compounds, sugars and fatty acids

(detected also by silylation/GC-MS). Beyond the GC-amenable fraction, two bimodal distributions centered 530 and 830 Da and tailing band 2-10 kDa confirms the presence of a higher amount of high molecular weight compounds (HMW). Performing quantitative analysis of HMW and assuming 1.2 gCOD $g_{substances}^{-1}$ (as for polysaccharides) SEC-RID detected HMW accounted for 56 ± 12 gCOD L^{-1}. This figure was close to that calculated (by difference) for non-GC-detectable compounds in table 1, namely 41±5 gCOD L^{-1}, suggesting that most of this GC eluding fraction consisted in strongly polar (detectable through RID) HMW which were not volatile or thermally unstable under GC injection conditions.

Table 1: deteiled chemical characterization of HTWW used in the study.

	Concentration	**± Sd. Dev. (n=8)**
COD (gO_2 L^{-1})	189	±12
TOC (gC L^{-1})	78	±15
TN (gN g^{-1})	8.4	±2.5
TAN (gN g^{-1})	3.3	±1.3
VFA (gCOD L^{-1})	9.2	±2.5
acetic acid	6.9	±2.5
propionic acid	1.2	±0.3
isobutyric acid	0.1	±0.1
butyric acid	0.5	±0.01
isovaleric acid	0.3	±0.1
valeric acid	0.0	±0
hexanoic acid	0.2	±0.3
Sugars (gCOD L^{-1})	49.4	±17.5
Long chain fatty acids (gCOD L^{-1})	7.0	±2.5
Glycerol (gCOD L^{-1})	14.0	±5.6
Adipic acid (gCOD L^{-1})	0.0	±0.0
Other hydroxy-acids/ketons (gCOD L^{-1})	7.9	±2.6
Lactic acid (gCOD L^{-1})	16.1	6.3
NAH[c] (gCOD L^{-1})	9.9	2.8
Pyroglutamic acid (gCOD L^{-1})	10.1	3.7
GC non-ID (gCOD L^{-1})	18.8	5
Suspended solids (gCOD L^{-1})	0	0
Non-detectable organics (gCOD L^{-1})	40.7	5.5

[c]N-containing aromatic heterocycles

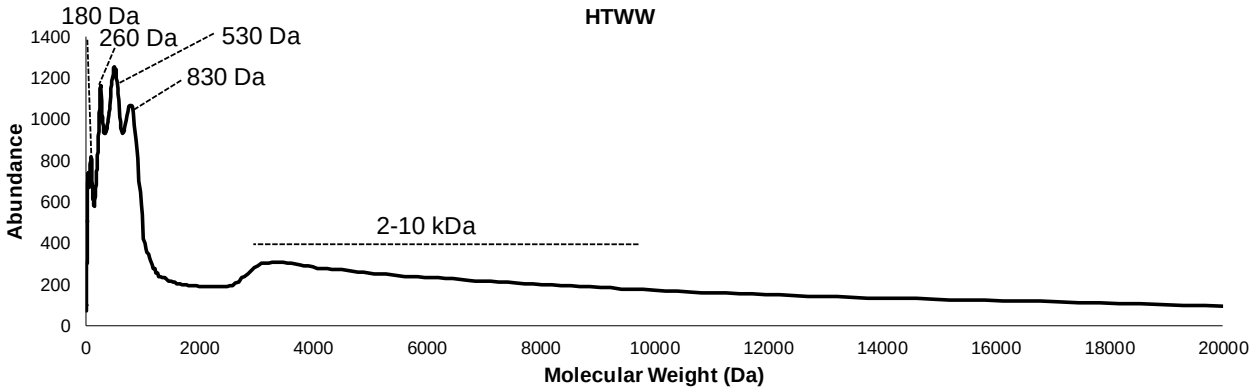

Figure 3: molecular distribution of polar dissolved organic matter in HTWW through SEC-RID analysis.

Observed composition of HTWW fall in broad range which are similar (if expressed in chemical classes) to that of HTWW found in literature for comparable substrates, namely organic waste HTL, microalgae HTL and sewage sludge HTL [7-9].

3.2 Long term reactor performance and steady state operations

The multistage biological system was continuously operated for 966 days. During this time period several changes to process configuration and conditions occurred which can be summarized into 8 main time spans (Figure 4):

1) the startup stage: two UASB reactors connected in series and fed with diluted HTWW from day 0 to 81 at an OLR at 0.1 gCOD L^{-1} d^{-1} and an HRT of 80 days (yields: 55% biogas, 10% VFA);
2) day 81 to 133: due to sudden increase of VFA concentration (over 5 gCOD L^{-1} VFA), HTWW input was stopped, recirculating the effluent liquid to maintains UASB hydrodynamics, until concentration of decreased to around 1 g L^{-1};
3) day 134 to 206: addition of a third UASB reactor, restart of regime operations and continuous monitoring of system (OLR ≈ 0.1 gCOD L^{-1} d^{-1}, HRT 120 days; yields 80% biogas, 10% VFA);
4) day 207 to 230: addition of the fourth UASB reactor (OLR ≈ 0.6 gCOD L^{-1} d^{-1}, HRT 16 days; yields 20% biogas, 35% VFA);
5) Day 231: after first evaluation of trends, some key system modification was performed. New system with a fifth UASB reactor, aerobic reactor and inlet dilution through recirculation (as in figure 1) was implemented. HTWW was diluted using recirculation of aerobic stage (AR) effluents (1:10 ratio) and provided to the anaerobic system.
6) day 232 to 708: steady state condition was achieved operating the system with constant conditions and variable ORL, HRT was set to about 20 and 10 days in anaerobic and aerobic reactors respectively, corresponding to a total organic residence time (in the whole system considering the looping due to OUT recirculation) equal to 300 days; (average yields equal to 10% biogas and 35%

for VFA); during this stage, analytical monitoring of the effluents was undertaken between day 654 and day 684.

7) day 708 to 880: tentative feed of more diluted HTWW without recirculation of OUT, further increase in VFA production (from 28% to 70%) decrease in biogas production (from 10% to 0%).

8) Day 881 to 966: following poor methanogenic performance, the system was switched back to HTWW with 1:10 dilution with OUT (as from day 361 and 707), OLR was brought to 0.8-1 gCOD L^{-1}. Consequentially, a reduction in VFA production (from 70 to 20%), and an increase in biogas production (from 0 to 80%) was observed.

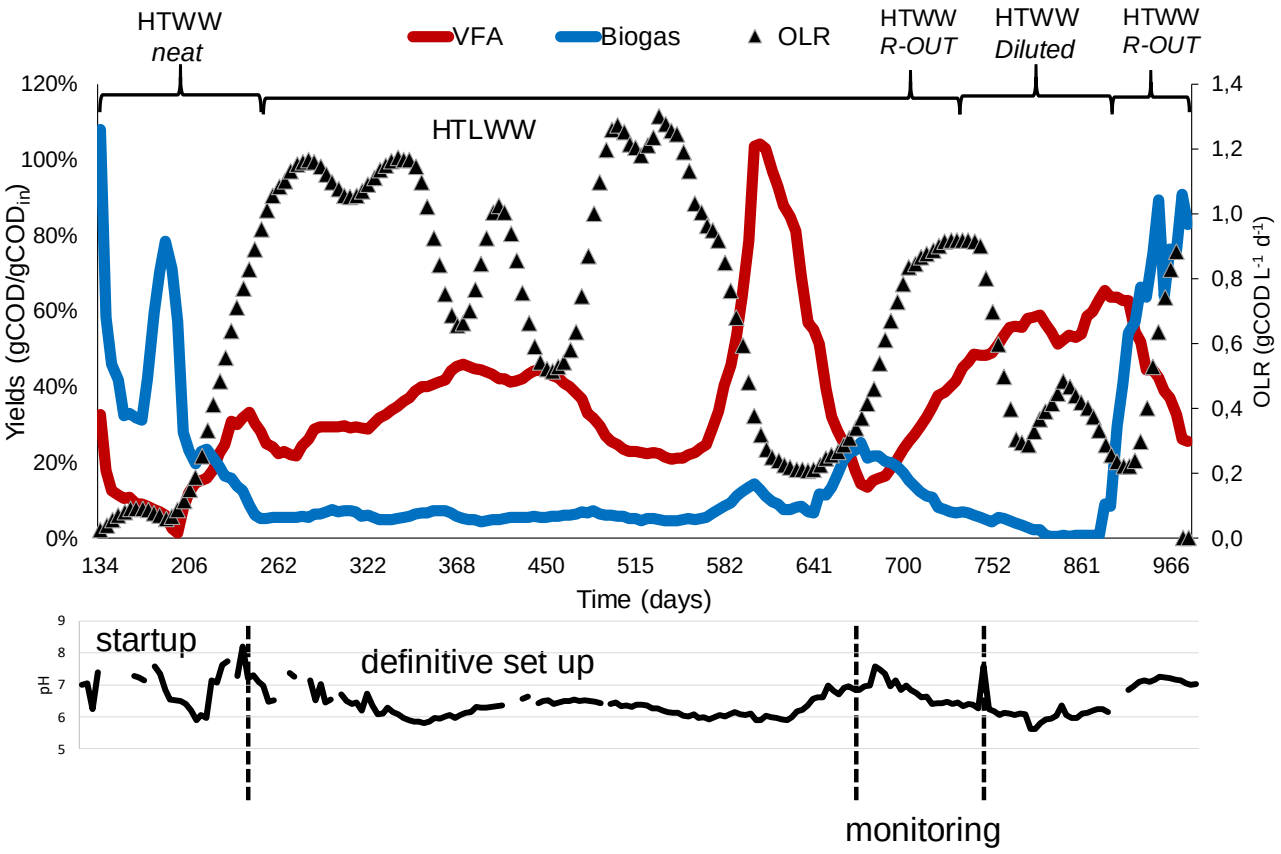

Figure 4: Long term reactor performance of multistage anaerobic digestor, biogas and VFA yields, pH trend and OLR variations. OUT-R: outlet effluent used for dilution of HTWW

The methanogenic (methane yield) and acidogenic (VFA yield) performance of the multistage system during long term experiment is shown in Figure 4. During the first 200 days, a quite good biological conversion of COD was observed with maximum yields of 80% and 35% for biogas and VFA, respectively. With the increase in the OLR (day 206), the system underwent a shift with an increase in VFA production (from 10 to 50% yields) and a drop in pH (which reached 5.9 at day 280) kept constant for more than 200 days, at the same time a reduction in biogas production was observed with constant yields around 10%. The subsequent increase in the OLR (day 500) with neat HTWW halted methane production and induced a

295 significant decrease in VFA yield. This probably suggests that such a steep change in ORL with neat HTWW
296 (189 gCOD L^{-1}) stream entering first UASB heavily inhibited all biological activity. To recover at least
297 acidogenic activity, ORL was slightly decreased obtaining back an increased acidogenesis which provided an
298 apparently quantitative yield of VFA for about one month. The further reduction of the OLR (day 574) has
299 led to a decrease in VFA production (20% yields) and a slight increase in biogas production (20% yields for
300 50 days). Given the recovery in biogas production, the OLR has been increased in conjunction with the
301 transition to more diluted HTWW (day 707). Without internal recirculation, the administration of diluted
302 HTWW has led to an acidification of the system with constant yields of VFA between 30 and 70% and a
303 total reset in the production of biogas. Finally, the transition back to HTWW with OUT recirculation (OUT-R)
304 has led to the reduction in VFA production (from 60 to 20% yields) and a rapid increase in biogas
305 production (from 10 to 80% yields), which was similar to that observed at the beginning of the study.
306 Looking at the entire Anaerobic-Aerobic system, a large variability of anaerobic digestion performance in
307 term of VFA and methane yields was observed. Nonetheless, for all the duration of the study, VFA were the
308 main conversion products, due to acidification and methanogenesis inhibition (after day 200). VFA were
309 easily converted under the aerobic step with production of CO_2 and bacterial biomass.

310 ## 3.3 Detailed study on Fate of HTWW organics

311 As soon as a relatively steady state was obtained, the fate of HTWW organics was evaluated by means of
312 detailed analysis of aqueous streams in three sampling point of the system (Figure 1). GC-MS (for VFA)
313 silylation-GC-MS (for small polar organics and sugars) and SEC (for quantitative evaluation of HMW
314 compounds) were performed, on weekly basis, before (IN) and after anaerobic digestion (AA) and after
315 aerobic treatment (OUT). These analyses provided a description on how organics are affected or
316 transformed by multistage anaerobic reactor and aerobic biological treatment. Qualitatively, IN was just a
317 diluted solution of HTWW, showing same HTL derivatives. These derivatives typically decreased in AA in
318 which few HTL derivatives persisted, while some newly formed additional compounds arose (e.g. VFA and
319 adipic acid). Finally, OUT was largely depleted in GC-MS identifiable constituents. SEC-RID analysis of
320 soluble organics provided a clear and simplified picture on the changes in amount and molecular weight
321 distribution of compounds. To the purpose of evaluating the actual effect of biological degradation, it is
322 useful to compare the composition of IN, AA and OUT with a time lag roughly equal to HRT in between
323 sampling points. This comparison is shown in Figure 5, that reports SEC-RID data relative to IN sampled at
324 day 654, AA sampled at day 672 and OUT sampled at day 686. SEC-RID chromatogram on IN shows a
325 molecular weight distribution which is similar to that observed for HTWW with additional HMW in the 3-12
326 kDa range. Chromatogram of AA shows an almost total disappearance of low molecular weight (<1 kDa)
327 compounds, a significant decrease of HMW compounds in the 2-10 kDa range. Finally, OUT chromatogram
328 shows further decrease of polar organics with molecular weight less than 7.5 kDa, and almost negligible
329 changes above that ceiling. The overall picture shown by SEC-RID results suggests a significant

biodegradation/removal of polar organic compounds. In particular, the decrease involved mainly the compounds with lower molecular weight, whereas HMW fractions were less affected by both aerobic and anaerobic treatment and showed a significant accumulation in the system.

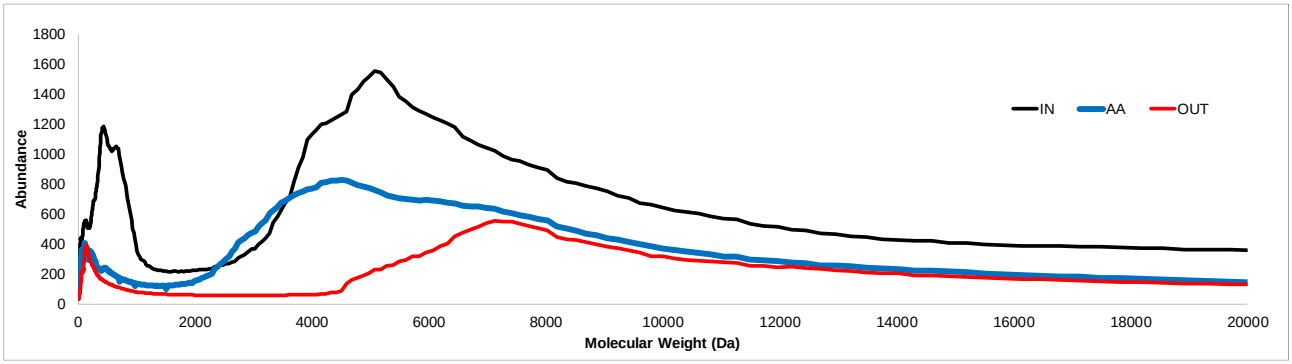

Figure 5: molecular distribution through SEC-RID analysis of polar dissolved organic matter in HTWW before and after biological treatments.

From a quantitative point of view, Figure 6 (A, B, C) details the time trends of some relevant organic compounds involved in HTWW treatment that were detected through silylation/GC-MS. Concentrations expressed as gCOD L^{-1}, are referred to the anaerobic digestion inlet (IN) and outlet (AA) and to the final aerobic stage (OUT). Most of the water-soluble substances with common natural analogs, for instance sugar derivatives, lactic acid or pyroglutamic acid were readily degraded in the anaerobic stage, whereas N-aromatic heterocycles showed a certain recalcitrance to biological degradation. In particular, hydroxypyridines, 2-pyrrolidinone and small hydroxy acids were scarcely degraded by anaerobic treatment, but were effectively degraded by aerobic treatment. However, 5-hydroxy-2-methylpyridine persisted in both anaerobic and aerobic treatment (AA and OUT). It is worth remarking that the final upward concentrations of 5-hydroxy-2-methylpyridine in AA was well above the toxicity ceilings for bacteria, [39] suggesting a significant concern for accumulation of this compounds in the system over long term.

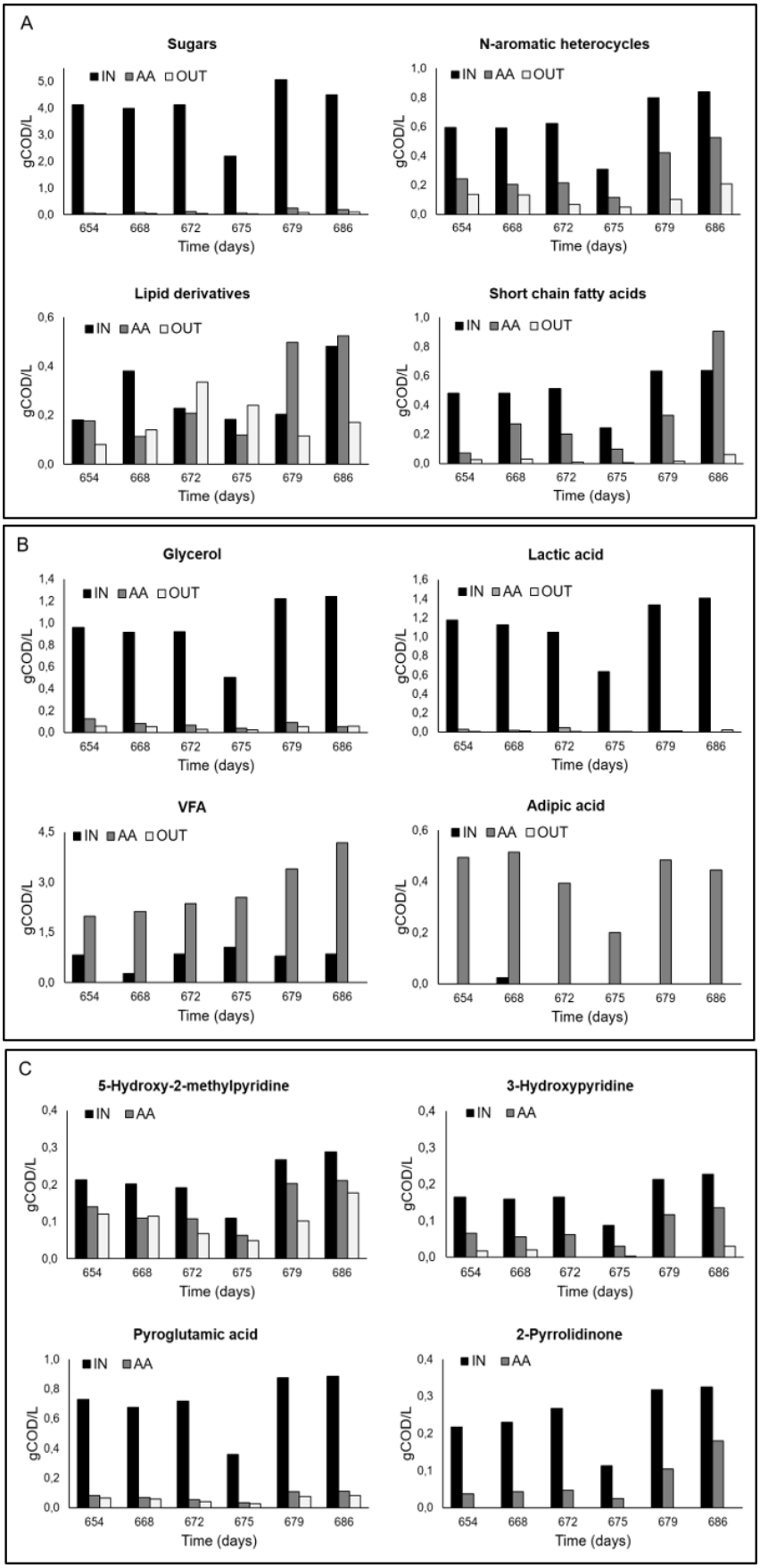

Figure 6: time trend of relevant organics in HTWW before and after biological treatment through silylation/GC-MS. A: sugars, N-aromatic heterocycles, lipid derivatives and short chain fatty acids; B: glycerol, lactic acid, VFA and adipic acid; C: focus on N-aromatic heterocycles.

To provide a general picture of biodegradation, Figure 7 summarizes the overall weighted average of major classes observed during the study in the three sampling points. IN solution consisted in HTWW organics (accounting for 19 gCOD L^{-1}) plus COD contribution from OUT used for HTWW dilution, mostly consisting in HMW compounds and SSV, accounting for remaining 4 gCOD L^{-1}. Entering COD was then 23 gCOD L^{-1}. After anaerobic treatment, the sum of soluble compounds in effluent of anaerobic reactor (AA) and methane generated was equal to 17 gCOD L^{-1}. Looking at the chemical composition in AA, about half of COD (9.1 gCOD L^{-1}) were found as fermentation products, namely methane (4 gCOD L^{-1}), VFA (3.7 gCOD L^{-1}), ammonia (1.1 gCOD L^{-1}) and adipic acid (0.6 gCOD L^{-1}), whereas the remaining COD was formed by identified unreacted HTWW organics (1.9 gCOD L^{-1}), suspended solids (1.8 gCOD L^{-1}), HMW (2.5 gCOD L^{-1}) and non-identified GC detectable constituents (2.1 gCOD L^{-1}). It is important to point out that, being the UASB completely anaerobic and under steady state, the overall decrease of COD observed between IN and AA, equal to 21%, is noticeable. The decrease could be almost entirely attributed to the precipitation of some organic constituents inside reactors and pipes surfaces. This hypothesis was assessed by means of inspections of some sections of anaerobic reactors, which shows a significant amount of bitumen like water-insoluble and dichloromethane soluble matter attached on the wall of reactor surfaces. Although precise assessment of the extent of this phenomenon was impossible without stopping the reactors, amount of precipitated matter was comparable to the COD loss observed between IN and AA. Moving to aerobic treatment, average COD of final effluent was equal to 7 gCOD L^{-1} which corresponds to a 60% decrease of total COD through aerobic respiration.

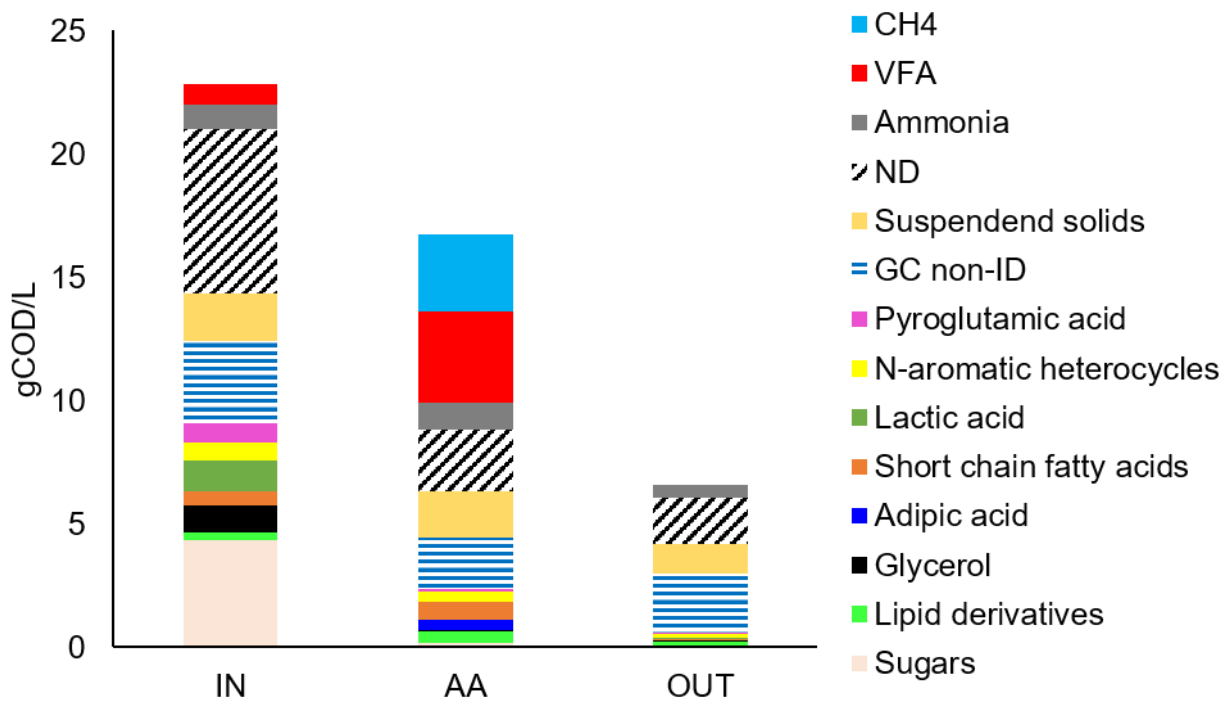

Figure 7: distribution of major chemical classes of HTWW before and after biological treatments. IN: average concentration of organics in HTWW diluted with effluent of aerobic stage; AA: average concentration of organics after anaerobic digestion; OUT: average concentration after aerobic stage.

Few identified unreacted HTWW organics (0.6 gCOD L^{-1}), mostly N-aromatic heterocycles, were detected in OUT, suggesting a general effectiveness of the process on the GC-MS amenable fraction. Most of compounds identified in HTWW were effectively degraded by combination of anaerobic and aerobic processes. OUT organic matter consisted in suspended solids 1.2 gCOD L^{-1} (mostly bacterial biomass, as assessed by microscope evaluation), 1.8 gCOD L^{-1} unconverted HMW and 2.4 gCOD L^{-1} of non-identified GC detectable matter that breakthrough the aerobic treatment. Such results are in accordance with SEC-RID analysis, which shows a negligible biodegradation of HMW.

3.4 Overall COD balance and process considerations

Figure 8 shows the COD balance between days 654-686 obtained by multiplying the observed COD concentrations by the volume of each stream flowed during monitoring, namely HTWW (about 2.8 mL/d), recirculation flow (R-OUT, about 23 mL/day) flow through AD (AA, about 26 mL/day) and treated aerobic effluent flow (OUT, about 2.6 mL/day). This balance allows to correctly evaluate the effectiveness of COD abatement of the system. The evaluation, performed on a time span equal to 32 days, being more than total HRT, can be considered quite adequate due to the fact that the system was already saturated and therefore the amount of organics that remain in the system at the end of the 32 days is fairly balanced by the organic matter already present in the system at the beginning. It is important to note that the balance strongly differs from concentration data especially for OUT, mainly due to the fact that internal recirculation, which brings back most of COD in OUT, is considered. Between day 654 and day 686, 16 gCOD

were added to the entire system as HTWW. Main constituents of this input were 4.4 gCOD sugars 1.4 gCOD lactic acid, 1.2 gCOD glycerol, 0.9 gCOD pyroglutamic acid, 0.9 gCOD as NAH, 1.6 gCOD unidentified GC-MS detectable compounds and 2.9 gCOD HMW compounds. Such COD merged with 4 gCOD from R-OUT, mostly formed by complex undefined residual organic matter and VSS (0.8 gCOD) prior to AD, which then received 20 gCOD in total. COD output measured in AA reveals that almost all identified organics but NAH were transformed in methane (2.6 gCOD, 0.91 L_{CH4}) and soluble fermentation products, namely VFA and adipic acid, whose amount was respectively 3.1, and 0.4 gCOD. VSS increase through AD which received 0.8 gCOD and produced 1.5 gCOD, suggesting bacterial growth or organics precipitation. Moreover, half the amount of unknown organic matter (non-identified GC-MS detectable and HMW) which enters in AD was not converted or was converted to other undefined organic matter. Aerobic treatment, which received AA as it is, was able to oxidize 65% of COD, producing a residual 5 gCOD, mainly formed by undefined organic matter, VSS and minor amount of NAH (0.1 gCOD). Such performance is in line with the typical oxidation yield observed for aerobic treatment with high HRT (10 days) and long residence time of sludge (equal to HRT). 35% of COD of AA, which survives the aerobic treatment, was mostly recirculated to AD (4.4 gCOD) and discharged as process effluent (0.5 gCOD). Mainly due to recirculation of OUT, the COD removal performance of the system could be considered excellent with 97% abatement observed during the time span of the study. Nonetheless most of this COD reduction is achieved by aerobic treatment at long residence time, with minimal methane production.

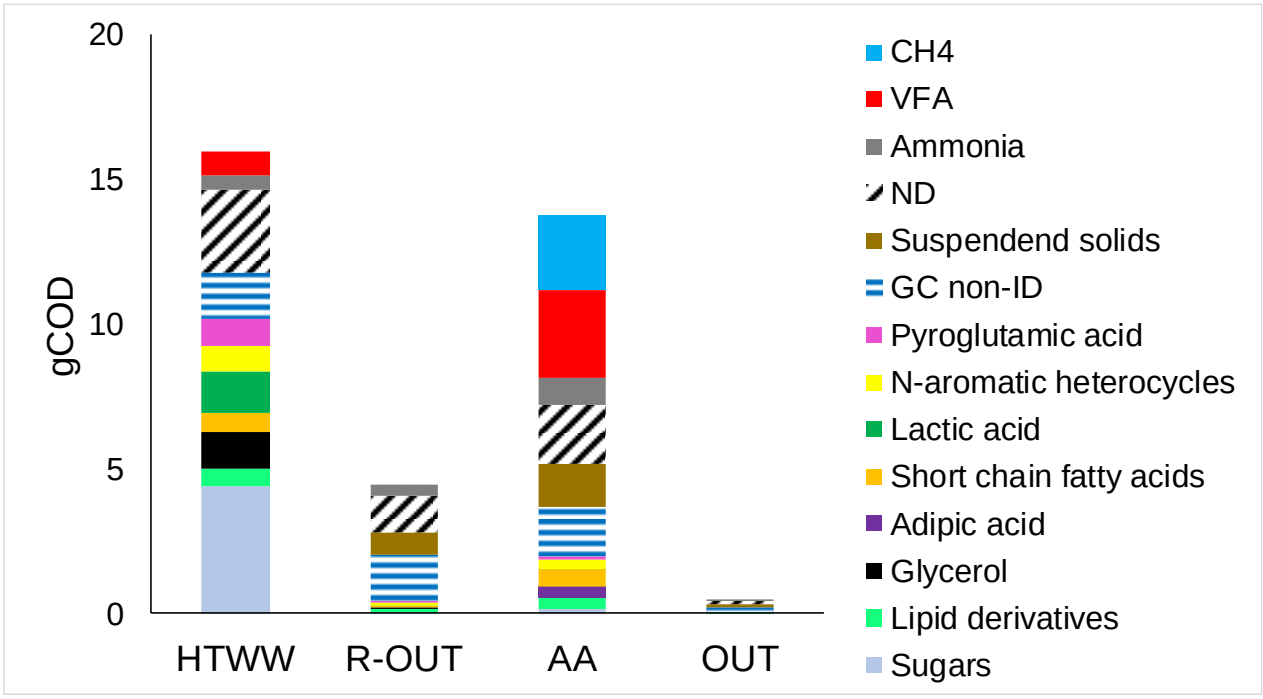

Figure 8: COD balance of major components of HTWW before and after biological treatments. HTWW: hydrothermal liquefacton watewater organics; R-OUT: recirculated outlet organics ; AA: output of organics after anaerobic digestion; OUT: output of organics after complete treatment

To evaluate the absolute performance of the system as functional unit for HTWW treatments, overall yields and productivities could be calculated. In 32 days of regime operation, 0.5 gCOD d^{-1} were provided to the system (400 mL AD and 200 mL aerobic reactor), which corresponds to an average organic loading rate equal to 0.83 gCOD L^{-1} d^{-1}. Looking at net balance, combined anaerobic and aerobic treatment processing of such input yielded to 0.08 gCOD d^{-1} of methane, 0.21 gCOD d^{-1} of insoluble matter (precipitated as oily substances in the AD) and, in output stream, minor amount of soluble organic matter and excess VSS. Most of COD, namely 0.20 gCOD d^{-1} is oxidized to CO_2 in the aerobic stage. Calculating the volumetric productivities, this means that the system was able to remove 0.8 gCOD L^{-1} d^{-1} with a volumetric productivity of methane 0.13 gCOD L^{-1} d^{-1} which corresponds to 45 L^{-1}_{CH4} m^{-3} d^{-1}. Assuming (as suggested by microscope evaluation) that most of suspended solids (1.8 gCOD L^{-1} and 1.2 gCOD L^{-1}) are in fact microorganism, specific activity was equal to 0.08 $gCOD_{CH4}$ $gCOD_{VSS}^{-1}$ or 0.5 gCOD $gCOD_{VSS}^{-1}$.

Table 2 summarizes the studies performed in this field to the objective of allowing a consistent comparison with literature. Data were obtained from back calculation of published experimental results from various configurations, highlighting the key AD performances obtained from biological treatment (with or without pretreatment), namely biomethane yields, volumetric productivities (in term of COD abatement or biomethane productivity) and, whereas available, specific activity of microbial consortia. Data concerning methane productivity are in line with results obtained by diluting the HTWW to the same concentration of this work (20 gCOD L^{-1}), which shows a volumetric productivity less than 1 gCOD L^{-1} d^{-1}. From the point of view of COD removal, the order of magnitude of the performance is comparable with that obtainable on highly diluted HTWW but without the need of dilution. Looking at specific activity of microorganism, this is 0.08 $gCOD_{CH4}$ $gCOD_{VSS}^{-1}$ for methane production or 0.5 gCOD $gCOD_{VSS}^{-1}$ for COD abatement. Methanogenic activity is one order of magnitude less than the values observed for commercial anaerobic digester operating on non-inhibiting streams, and very close to that obtained for optimized biomethanation of diluted HTWW. On the other hand, although there are scarce data on aerobic degradation of HTWW, we can point out that overall COD abatement rate is in the lower boundary of aerobic wastewater system, suggesting that non-methane pathway (HTWW to VFA then to CO_2) could be less sensitive to HTWW toxic constituents. As a whole, the comparison with the literature suggests that internal recirculation of aerobically treated effluent can be used instead of water to obtain a lower concentration of COD without additional adverse impact. According to this it is interesting to notice that, a higher recirculation rate (e.g. 1:20, to bring IN COD to 10 gCOD L^{-1}) could improve the methane productivity. Nonetheless, this option should not necessarily improve the COD removal rate and could bring additional issues. In particular, aerobic system should work at room temperature (due to low solubility of oxygen at high temperature) and AD should work at thermophilic or mesophilic temperature. Therefore, the increase of recirculation rate increases the heat need of the system or the size of heat exchanger (to heat up the recirculation flow to AD temperature). Another possible improvement is related to bacterial concentration in AA, which was

relatively low (1.8 gCOD L^{-1}), at least in comparison with other conventional UASB configuration [40]. Since the system was at equilibrium (growth is balanced by the washout) and given the nutrients profile of HTWW, this phenomenon should not be related to bacterial growth or linked to the feedstock provided but, more probably, to the configuration used in this work. Therefore, a promising strategy could be to increase the amount of bacterial biomass in the anaerobic digester by means of different reaction configurations or filling materials. This strategy could potentially improve the volumetric productivity of AD by one order of magnitude without adverse effects, meanwhile determining a further reduction of COD of effluent after aerobic treatment. Finally, detailed analysis performed in this work clearly shows that, even when methanogenesis is heavily inhibited, high concentration of VFA can be obtained by acidogenesis. Although this study was not optimized to VFA production, overall yields were significant, close to 80% $gCOD_{VFA}$ $gCOD^{-1}$ in certain stages of the experiments. Final concentrations of VFA were in the self-inhibition range for acidogenesis (14-12 gCOD L^{-1} and slightly acidic pH) for most of the study, which means that observed VFA yields can be less than the maximum achievable yields. As a proof of concept, pre-diluted HTWW was fed to the system after day 708 in order to assess the maximum yield of VFA. Under these conditions, yields of VFA increased up to 68% suggesting that self-inhibition of acidogenesis could be removed by continuous recovery of VFA (instead of dilution). This finding is also in agreement with observations from other authors who found a quite relevant VFA production even under conditions able to inhibit methane production [20,21]. VFA, being a common chemical or biological intermediate, can be separated and used for several purposes [41,42]. VFA enriched solution can be provided to other microorganisms to obtain polyhydroxyalkanoates or microbial oils [43]. VFA can be chemically converted to drop-in chemicals like hydrocarbons, [44] ketones [45] and alcohols [46]. All these evidences suggest that in place of exploiting the slow growing and sensitive Archaea to obtain methane, different approach targeted to VFA through non-methanogenic routes could represent a challenging but promising research area.

*Table 2: typical performances of biological treatment according to previous literature studies about HTWW valorization. SE: solvent extraction; AC: activated carbon; PUf: polyurethane foam.*this study.*

feedstock	PRT	HTWW $\frac{gCOD}{L}$	COD $\frac{gCOD}{L}$	VFA $\frac{gCOD}{L}$	Yield $\frac{gCOD}{gCOD_{in}}$	VP $\frac{gCOD}{L \cdot d}$	HRT d	Reactor	Ref
rice straw			0.8		51%	0.04	27	batch	[14]
rice straw	SE		0.8		65%	0.10	27	batch	[14]
rice straw			16		44%	1.40	5	ASBR	[14]
rice straw	SE		16		62%	1.99	5	ASBR	[14]
swine manure			3.4		64%	0.05	65	batch	[15]
swine manure			27		22%	0.10	65	batch	[15]

swine manure	AC		34		64%	0.53	65	batch	[15]
mixed microalgae			3.3		56%	0.13	45	BMP	[16]
mixed microalgae			3.2		44%	0.10	45	BMP	[16]
mixed microalgae			3.3		61%	0.55	45	BMP	[16]
nannochloropsis sp.		62	1.0		15%	0.01	13	BMP	[17]
nannochloropsis sp.	$MgCl_2$	51	1.0		52%	0.06	13	BMP	[17]
nannochloropsis sp.		91	1.0		12%	0.03	5	BMP	[17]
nannochloropsis sp.	AC	43	1.0		30%	0.07	5	BMP	[17]
spirulina		89	17		33%	0.05	140	2xBatch	[18]
spirulina	GAC	89	17		31%	0.05	140	2xBatch	[18]
spirulina	Zeolite	89	17		38%	0.06	140	2xBatch	[18]
spirulina	PUf	89	17		41%	0.08	120	2xBatch	[18]
Chlorella 1067		76	4.0		50%	0.18	50	BMP	[19]
Chlorella 1067		76	7.0		12%	0.06	45	BMP	[19]
Chlorella 1067	Zeolite	71	4.0		60%	0.40	50	BMP	[19]
Chlorella 1067	Zeolite	71	7.0		14%	0.17	45	BMP	[19]
Tetraselmis AGT		87	19		89%	0.74	18	CSTR	[20]
Tetraselmis AGT		87	61	8.6	0%	0.37	23	CSTR	[20]
Chlorella AGC		75	20		69%	0.56	18	CSTR	[20]
Chlorella AGC		75	53	9.6	4%	0.55	18	CSTR	[20]
swine manure			10	1.6	39%	0.07	50	BMP	[21]
swine manure	AC		10		65%	0.17	50	BMP	[21]
swine manure	O_3		10	0.2	53%	0.17	50	BMP	[21]
swine manure			10	4.5	7%	0.33	14	BMP	[21]
sewage sludge		60	10		58%	1.44	4	ASBR	[22]
sewage sludge	AC	60	10		74%	1.85	4	ASBR	[22]
sewage sludge		80	0.8		49%	0.05	10	BMP	[23]
sewage sludge		77	0.8		53%	0.06	10	BMP	[23]
spirulina		143	4.7		76%	0.01	35	BMP	[24]
spirulina	H_2O_2	143	3.8		70%	0.01	35	BMP	[24]
swine manure		40	5.0	0.7	63%	0.22	35	BMP	[25]

swine manure		40	20	9.6	15%	0.06	100	BMP	[25]
swine manure	O_3	40	20	6.6	32%	0.14	100	BMP	[25]
swine manure	AC	40	20	2.5	61%	0.33	100	BMP	[25]
swine manure	O_3+AC	40	20	2.5	58%	0.36	100	BMP	[25]
food waste		4.0	1.0		48%	0.06	30	BMP	[26]
food waste		4.0	1.0		43%	0.01	30	BMP	[26]
sewage sludge			10	1.2	60%	1.49	4	UASB	[27]
OFSW		189	189	0	16%	0.8	20	UASB	*
OFSW		189	189	0	97%	0.8	220	UASB+AR	*

4. Conclusions

Continuous biological processing of hydrothermal liquefaction wastewater was demonstrated for the first time, without any external input and for a long time of almost 3 years. Such a configuration allowed firstly to convert most of HTWW organics into VFA and methane and, after aerobic treatment, to reduce significantly the HTWW COD. Anaerobic treatment yielded mostly VFA and other fermentation intermediates, with minor methane yield. The overall yield of fermentation products in the last part of the study was about 50%, which suggests a significant biodegradability of the HTWW. A total COD reduction equal to 97% was achieved through an anaerobic fermentation coupled to aerobic treatment of effluent. The presence of recalcitrant nitrogen containing aromatic compounds, confirmed to be the key challenge for improving methane yields and system productivities. In moving from wastewater disposal (achievable with anaerobic-aerobic systems) to wastewater valorization into biogas, the effort should be focused to process intensification as the selective removal of biological inhibitors by pretreatment of HTWW. Pathways alternative to biogas generation, such as the production/purification of VFA is worth of consideration in future studies

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List of Abbreviations

AA: Sampling point after the anaerobic digestion (Figure 1)

AC: Activated carbon

AD: Anaerobic digestion

AR: Aerobic Reactor

COD: Chemical Oxygen Demand

CSTR: Continuously Stirred Tank Reactor

DAD: Diode Array Detector

GAC: Granulated activated carbon

GC-MS: Gas chromatography coupled with mass spectrometry

HHV: Higher Heating Value

HMW: High Molecular Weight substances

HPLC: High Pressure Liquid Chromatography

HRT: Hydraulic Residence Time

HTL: Hydrothermal Liquefaction

HTWW: Hydrothermal Treatment Wastewater

iCOD: Insoluble Chemical Oxygen Demand

IN: Sampling point before the anaerobic digestion (Figure 1)

NAH: nitrogen containing aromatic hydrocarbons.

OLR: Organic Loading Rate

OMSW: Organic Fraction of Municipal Solid Waste

OUT: Sampling point after the aerobic stage (Figure 1)

PUf: Polyurethane foam

RID: Refraction Index Detector

R-OUT: amount of recirculated organics (as gCOD) from OUT

sCOD: water soluble COD

SE: Solvent Extraction

silylation/GC-MS: silylation and analysis with GC-MS

tCOD: Total COD

TN: Total Nitrogen

TSS: Total Suspended Solids

UASB: Upflow Anaerobic Sludge Blanket

VFA: Volatile Fatty Acids

VSS: Volatile Suspended Solids

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