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**Valorisation of the liquid fraction from hydrothermal carbonisation of sewage
sludge by anaerobic digestion**

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Abstract

BACKGROUND: The mesophilic anaerobic digestion of the liquid fraction from hydrothermal carbonisation (208 °C, 1 hour) of dehydrated sewage sludge, has been studied. Two initial inoculum concentrations (IC) (10 and 25 g COD/L) and four inoculum to substrate ratios (ISR) (2, 1, 0.5 and 0.4 on a COD basis), have been selected to analyse their influence on the evolution of the anaerobic digestion process.

RESULTS: The substrate is characterised by a high COD (95.5 g/L) and TKN (8.7 g N/L) values. High inoculum concentration (25 g COD/L) and/or low ISR (≤ 0.5) inhibited the methanogenesis due to the high ammonia nitrogen (1.4 g TAN/L) and VFA (>4 g COD/L) released. For the inhibited samples final COD removals lower than 15% and IA/TA ratios higher than 0.3 were found. The greatest methane yield, (177 \pm 5 mL CH₄ STP/g COD_{added}) was achieved at 25 g COD/L of IC and at an ISR of 2.

CONCLUSION: During the anaerobic digestion of the liquid fraction from the hydrothermal carbonisation of sewage sludge, the IC and ISR must be adequately selected for a proper operation of the process and a successful valorisation. According to the results, working at an $\text{ISR} \geq 1$ is recommended.

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1. INTRODUCTION

Wastewater treatment plants (WWTP) generate huge amounts of sewage sludges which are currently managed through agricultural application, incineration or landfilling. In this context, different methods of thermal valorisation, such as pyrolysis or gasification, are being investigated in the last two decades.¹ As an alternative to these methods, hydrothermal carbonisation (HTC), a relatively new process for biomass carbonisation, usually performed at 180–375 °C under auto-generated pressures,² has been gaining attention, because energy-intensive predrying is not necessary.³ Moreover, the hydrochar (HTC char) has a higher heating value compared to the biochar produced from slow-pyrolysis or conventional carbonisation at the same temperature.⁴ Hydrochar has several industrial and environmental applications such as soil remediation, solid fuel and CO₂ sequestration.^{5,6} The char obtained via HTC is a slurry that needs to be separated through filtration. Most of the studies of HTC have been focused on the optimisation of the reaction conditions to obtain solid fuels.^{3,7} Depending on the process conditions, the HTC liquor can contain up to 15-20% of the initial carbon, mainly in the form of formic and acetic acids, sugars, nutrients and other compounds.^{8,9} Also, the formation of recalcitrant or inhibitory compounds such as furfural, phenols and furan may occur during the hydrothermal process.^{10,11} Therefore, that liquid fraction has much higher total chemical oxygen demand (COD_t) than most types of organic wastewaters. Besides, the HTC conditions (temperature and time) affect to that COD_t. Values around 60 g/L have been reported from HTC of food waste or orange pomace^{12,13} and somewhat lower values (40-50 g/L) have been obtained from agro-industrial residues like chaff, corn silage or thin stillage,^{14,15,16} while for HTC of primary sewage, mixture of primary and secondary sludge and digested municipal sewage sludge, values of 23, 52.5 and 34 g/L have been, respectively, reported.^{2,17,18} Therefore, the liquid fraction

from HTC, needs to be conveniently managed before final discharge and moreover its high organic load offers potential interest for the sake of valorisation. Different solutions have been proposed, including the use as feedstock for chemical production¹⁹ recycling in consecutive HTC runs to improve the carbon yield or biological stabilisation.^{9,20} Chemical and biological treatments have been evaluated for the liquid fraction from HTC of sewage sludge. Wet air oxidation allowed reducing total organic carbon (TOC) up to 60%.²¹ Ramke *et al.*²² reported COD reduction over 85% upon aerobic degradation. Anaerobic digestion of that liquid fraction has been suggested as a potential route to optimise energy recovery.^{23,24} However, the potential to produce methane from the liquid fraction of HTC has been scarcely studied and the existing information deals mainly with lignocellulosic residues.^{13,14,16,25} Qiao *et al.*¹⁷ determined the biogas and methane production of the supernatant obtained from HTC of municipal sludge. Danso-Boateng *et al.*² used experimental data of COD from the liquid fraction of the hydrothermal carbonisation of sewage sludge to estimate theoretical methane yields.

The aim of this study is to analyse the potential valorisation of the liquid fraction from hydrothermal carbonisation of sewage sludge by anaerobic digestion. For that purpose, the initial inoculum concentration (IC) and the inoculum to substrate ratio (ISR) have been checked as main variables. Two IC values (10 and 25 g COD/L) and four ISR (2, 1, 0.5 and 0.4, in terms of COD) were tested. So far, only few studies can be found in the literature relative to the effect of those two variables on the evolution of singular parameters of anaerobic digestion: pH, volatile fatty acids (VFA), alkalinity, ammoniacal nitrogen, COD and methane potential. The anaerobic digestion experiments were performed in batch-wise.

2. EXPERIMENTAL

2.1 HTC experiments and substrate characteristics

A sewage sludge-derived solid (SSDS) with 85% moisture was collected from a full-scale membrane bioreactor treating industrial wastewaters from a cosmetics factory. It was maintained at -20 °C until use. HTC was performed at 208 °C for 1 h in a ZipperClave® pressure vessel electrically heated using 1.5 kg of SSDS. The final temperature was reached at a heating rate of 3 °C/min. The reaction was stopped by cooling in a heat exchanger using tap water. The liquid fraction was recovered by centrifugation (3500 rpm for 1 h) and filtration (0.45 µm) being then maintained at 4 °C until batch anaerobic digestion were performed.

The main characteristics and composition of this liquid fraction from HTC were as follows (average values of three determinations with standard deviations): pH: 5.1±0.1, soluble COD (CODs): 95.5±0.4 g O₂/L, total solids (TS): 51.9±0.5 g/L, volatile solids (VS): 46.2±0.5 g/L, biochemical oxygen demand (BOD₅): 25.6±1.1 g/L, TOC: 42.6±0.9 g/L and total Kjeldahl nitrogen (TKN): 8.7±0.1 g N/L. The analysis by HPLC/RI allowed determining the concentration of formic, acetic, iso-butyric and butyric acid, which yielded values of 1420±20 mg/L, 2269±33 mg/L, 930±11 mg/L and 94±4 mg/L, respectively.

2.2 Inoculum source

The inoculum was a granular anaerobic sludge from an industrial digester treating brewery wastewater under mesophilic conditions (35 °C). Its main characteristics were: pH: 7.6 ± 0.1 , TS: 61.9 ± 0.9 g/L, VS: 55.7 ± 0.9 g/L, CODt: 91.2 ± 1.4 g O₂/L and TKN: 2.2 ± 0.1 g N/L.

2.3 Experimental set-up and procedure

Anaerobic digestions runs were carried out in 120 mL glass serum vials, filled with 60 mL of a suspension of inoculum, substrate and a basal medium with macronutrients (NH₄Cl, 280 mg/L; K₂HPO₄, 250 mg/L; MgSO₄·7H₂O, 100 mg/L; CaCl₂·2H₂O, 10 mg/L; yeast extract, 100 mg/L) and micronutrients (FeCl₂·4H₂O, 2 mg/L; CoCl₂·6H₂O, 2 mg/L; 0.5 MnCl₂·4H₂O, mg/L; AlCl₃·6H₂O, 0.09 mg/L; (NH₄)₆Mo₇O₂₄·4H₂O, 0.05 mg/L; H₃BO₃, 0.05 mg/L; ZnCl₂, 0.05 mg/L; CuCl₂·2H₂O, 0.038 mg/L) as it is recommended (Holliger et al.²⁶). Before sealing the vials with rubber stoppers and metallic crimps, the suspensions were flushed with N₂ for 3 min. The vials were placed in a static incubator at mesophilic temperature (35±1 °C) and were daily mixed. Table 1 describes the experimental conditions used in these batch anaerobic digestion experiments. As indicated before, two IC values (10 and 25 g COD/L) and four different ISRs (0.4, 0.5, 1 and 2, on a COD basis) were tested. The experimental period was extended until the methane production was undetectable or less than 5% of the total produced (on the last day).

For every inoculum concentration three blank runs (for subtracting the methane production due to biomass decay and the possible presence of residual substrate in the

inoculum) and three positive controls (tests with starch as only substrate) were carried out (these control yielded approximately 350 ml STP $\text{CH}_4/\text{g COD}_{\text{added}}$). For each of the 8 conditions tested (Table 1), 9 batch reactors were ran. Six of them were sacrificed and removed every one or two days initially and then weekly in order to study the time-course of the anaerobic digestion process. The other three reactors were used only for biogas analysis (volume and composition).

2.4 Analytical methods

The inoculum was characterised by measuring the pH (using a model Crison 20 Basic pH-meter), TS and VS, according to the standard methods 2540B and 2540E, respectively.²⁷ The COD_t was determined by the method proposed by Raposo et al.²⁸. TKN was determined acidifying 1000 mg of sample with 15 mL of concentrated H_2SO_4 (85% wt). In addition, 5 g of catalyst [(Cu–Se) (1.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ + 2% Se)] were added, and the sample was digested sequentially in a thermoblock for 15 min at 150 °C, 15 min at 250 °C and 90 min at 390 °C and then distilled and titrated according to the standard method 4500E.²⁷

The liquid fraction from hydrothermal carbonisation, as well as the sacrificed samples (centrifuged and filtered through a 0.45 μm filter) were used to determine the following parameters: pH; partial and total alkalinity (PA and TA, by pH titration to 5.75 and 4.3, respectively;²⁹ intermediate alkalinity (IA, defined as the difference between TA and PA); CODs (using the closed digestion and colorimetric standard method 5220D;²⁷ TOC (measured with an automatic analyser TOC-VCPN, Shimadzu); TKN; total ammonia nitrogen (TAN), determined by distillation and titration according to the

standard method 4500E;²⁷ free ammonia nitrogen (FAN, according to Hansen et al.³⁰; individual VFA were quantified by HPLC coupled with a refraction index detector (HPLC/RI, Varian, Agilent Technologies, Santa Clara, CA, USA) using a sulfonated polystyrene resin in the protonated form (67H type) as the stationary phase (Varian Metacarb 67H 300 mm) and sulfuric acid (0.0125 mol/L in milliQ water) as the mobile phase at a flow rate of 0.8 mL/min. Column temperature was 65°C.³¹ Gas chromatography/ion trap mass spectrometry (GC–MS; CP-3800/Saturn 2200 using a Varian CP-8200 autosampler injector, and a solid phase microextractor, Carbowax/Divinylbenzene Yellow-Green to identify chemical species). The capillary column used was a Factor Four VF-5ms (30m long, 0.25mm diameter). Sample injection was carried out with split-less at 220 °C, using He as carrier gas. The temperature program used in the GC/MS analyses ramped as follows: 40 °C for 15 min and 15 °C/min until 250 °C. The compounds were assessed using the NIST 2008 Library.

Biogas and methane production were measured once every day during the first 3 days and eight more times for the rest of the incubation period. Biogas production was determined by manometric method (Rozzi and Remigi, 2004), measuring the pressure increase in each vial by an electronic pressure monitor (ifm, PN 7097). It was expressed at standard temperature and pressure (STP: 273K, 1bar) conditions. Biogas was subsequently exhausted to re-establish atmospheric pressure. Methane production was calculated by subtracting the amount of methane produced in the blank controls from the methane production of each batch reactor. The gas composition (H₂, CO₂ and CH₄) was determined by gas chromatography using a Bruker 450-GC (Goes, The

Netherlands) coupled with a thermal conductivity detector (TCD) for H₂ and CO₂ and a flame ionisation detector (FID) for CH₄.³¹

3. RESULTS AND DISCUSSION

3.1. Anaerobic digestion process

The initial and final values of pH, total alkalinity and IA/TA ratio from the anaerobic digestion experiments are collected in Table 2. The initial pH was higher than 7 for samples with an ISR ≥ 1 , but lower for the rest, even lower than 6 for samples 2.5-0.5 and 2.5-0.4. The pH of the HTC liquid fraction was acidic (5.1 ± 0.1), in agreement with the observed by other authors.^{22,25} In all the cases the pH increased during the anaerobic process. It has been stated that values lower than 6.5 can provoke methanogenic inhibition.³³ The initial total alkalinity for the tests developed at 10 g COD/L of IC value, ranged between 1 and 2 g CaCO₃/L, being significantly higher for the runs at higher concentration (25 g COD/L). The final alkalinity values increased around twofold in all cases, from 2.2 (run 1-2) to 9.50 (run 2.5-0.4) g CaCO₃/L. Alkalinity values above 2.5 g CaCO₃/L provide a buffering capacity, so that even a large increase of VFA reduces only minimally the pH.³⁴ Looking at those values it would seem that the anaerobic process performed stably and well buffered. However, except for the samples 1-2, 1-1 and 2.5-2, the starting intermediate to total alkalinity ratio was higher than 0.3, which is not recommended for a good stability of the anaerobic process.³⁵ Figure 1 shows the time-course of tVFA under the different experimental conditions tested. tVFA concentration decreased along the experimental period in 1-2, 1-1 and 2.5-2 runs. For the rest of the experiments tVFA concentration decreased during the first 1-2 days, due to the degradation of short-chain fatty acids present in the substrate, which

were easily degraded. Beyond that time the tVFA concentration increased until the 10th-15th day due to the acidification of the complex organic matter presents in the substrate and then remained more or less stable. As it is well known, the accumulation of intermediate products, such as VFA, is indicative of process unbalance. The amount of VFAs produced increased at increasing IC and decreasing ISR values, reaching values around 9 g/L (2.5-0.5, 2.5-0.4). Silvestre et al.³⁶ observed strong inhibition at VFA concentrations above 5 g/L, although the collapse of the system commonly occurs at around 10 g/L.³⁷ Therefore, it is clear that the pH cannot provide by itself information on imminent failure, because in medium or well-buffered solutions high VFA concentration can develop without appreciable pH decrease. Thus, direct measurements of VFA, alkalinity and in particular the IA/TA, are necessary.³⁸

The time-course of TAN is depicted in Figure 2, which includes also Tables with the initial TKN values. TAN reached above 80% of TKN in 1-2, 2.5-2 and 1-1 runs. The rest of experiments showed lower nitrogen hydrolysis (approximately 65-70%) and for the 2.5-0.4 condition only 46% took place. However, final TAN values for these experiments were above 1400 mg N/L. Ammonium represents an essential nutrient for microorganisms and contributes to the stabilisation of pH. Ammonium bicarbonate buffers the system allowing to operate even at high VFA concentrations. However, FAN is highly toxic, especially to acetoclastic methanogens.³⁹ Increasing the pH displaces the ammonium/ammonia equilibrium towards the second. At 35 °C and pH lower than 7, TAN is almost completely in the form of ammonium, and at pH 8 only around 10% is as FAN. The highest FAN concentration was observed in the 2.5-1 experiment, with 125 mg NH₃/L, substantially below than the 700-1100 mg NH₃/L range reported to cause strong inhibition.⁴⁰ In the case of TAN, inhibiting values

between 1500 and 7000 mg N/L have been reported.⁴¹ Thus, operational difficulties through inhibition of the methanogenic Archaea because of TAN accumulation may occur clearly at the highest IC value tested and at an $ISR < 2$ (experiments 2.5-1, 2.5-0.5 and 2.5-0.4), and also at the lowest IC when $ISR < 1$ (1-0.5 and 1-0.4)

The initial substrate concentration ranged from 5 to 62.5 g O₂/L of COD, with proportionally higher values as the digesters were more heavily loaded. Initially, the CODs values increased in each experiment up to 6.8 and 68.5 g O₂/L, respectively (Figure 3) as the result of inoculum hydrolysis. COD removal was negligible for 2.5-1 and 1-0.4 runs (2-4%), while the highest reductions were observed in the 1-2, 1-1 and 2.5-2 experiments (38-44.5%). Final COD attributable to VFA was less than 20% for the experiments carried out at an $ISR \leq 1$. Therefore, above 80% of the final COD in those experiments is due to other refractory compounds which accumulate in the reactor inhibiting the anaerobic process.

Phenolic and aromatic compounds were identified in the starting HTC liquid. This is consistent with a common carbonisation route including hydrolysis, dehydration, decarboxylation, condensation, polymerisation and aromatisation reactions as previously reported by Danso-Boateng et al.² Products such as aldehydes, furans, pyrroles, pyrazines, and pyridines were also detected. The high concentration of TKN can be due to the presence of several nitrogen-containing species that may have relatively high molecular weight. Anaerobic digestion led to almost complete removal of the furan (furan, 2,5-dimethyl-) and aldehyde (4-methoxycinnamaldehyde) species present in the initial HTC liquid, but a new aldehyde compound (2,3,6-

trichlorobenzaldehyde) appears as a degradation intermediate in the experiments at the lowest IC value (1-2 and 1-1) (Table 3). Phenols and other oxygenated aromatics compounds were partially removed. Whereas, most pyrazines and aromatic amines (pyrazine, 2-ethyl-5-methyl-; 2,3-diethylpyrazine; pyrazine, 2,5-dimethyl-3-propyl-; benzenamine, 3-methoxy-; 4,5-dimethyl-ortho-phenylenediamine) were refractory. Some compounds (1H-indole, 7-methyl-) not found in the initial substrate were detected in the final samples after anaerobic digestion, which may correspond to refractory intermediates. The presence of refractory species, may affect to the methane yield by limiting the efficiency of the biological process.^{42,43} The presence of enough microorganisms may restrain the inhibitory effect of such species, thus explaining the higher methane production at the highest ISR.

3.2. Methane potential yield

Figure 4 shows the cumulative methane yield along the anaerobic digestion experiments. Methane production began immediately in assays 1-2, 1-1 and 2.5-2, reaching final figures of 144 ± 1 , 158 ± 4 and 177 ± 5 mL CH₄ STP/g COD_{added}, respectively. These yield values are lower than the observed for the anaerobic digestion of the liquid fraction from HTC of agricultural residues like thin stillage or orange pomace, where 300 and 213 mL CH₄ STP/g COD, were reported, respectively,^{16,13} or mixed sewage sludge, with 257 mL CH₄/g COD,¹⁷ but fairly similar to the obtained by Weiner et al.¹⁴ for the liquid fraction from HTC of chaff using organosolv as the liquid source for carbonisation (174 ± 9 mL CH₄/g COD) or by Wirth et al.¹⁸ for the liquid fraction from HTC of digested sewage sludge as sole substrate operating in continuous feed mode (120-180 mL CH₄ STP/g COD_{added}). For all the other conditions tested,

inhibition of the methanogenic stage occurred, suggesting that not only the ISR but also the IC affect to methane production. At an ISR below 1 (runs 1-0.5, 1-0.4, 2.5-0.5, 2.5-0.4) and even at 1 for the highest IC (2.5-1), overload takes place, due to the increased availability of easily hydrolysable material in the reactor, which in turn leads to VFA and TAN accumulation, low COD removal, IA/TA ratios ≥ 0.75 and ultimately methanogenesis inhibition. ISR of 0.5 (on a VS basis) have been reported to inhibit methane production in anaerobic digestion of winery waste, microcrystalline cellulose production wastewater and piggery slaughterhouse.^{42,44,45}

The results of the three experiments giving significant methane yields (1-2, 1-1 and 2.5-2), were fitted to a first-order rate equation which is a simple and useful model that has been frequently applied to anaerobic digestion systems.^{42,43,44} The basic equation is:

$$G = G_m \cdot [1 - \exp(-k \cdot t)] \quad \text{Eq (1)}$$

where G represents the cumulative methane yield at a time t , G_m is the ultimate methane yield of the substrate analysed and k the specific rate or apparent kinetic constant. The ultimate methane yield corresponds to the final value when no more gas is released from the reactor. This equation has been frequently applied to anaerobic digestion.⁴⁶ Samples of $\text{ISR} \leq 0.5$ showed almost complete inhibition and therefore were not considered.

Origin software (version 8.0) was used to fit the experimental data to equation (1). Table 4 collects the values obtained for k and G_m (95% confidence), as well as the corresponding determination coefficient (R^2). This simple kinetic approach allows a good prediction of the experimental results, as can be seen in Figure 4. The values of the apparent kinetic constants are related to the concentration of the inoculum. The

highest ones ($0.048 \pm 0.008 \text{ d}^{-1}$ and $0.043 \pm 0.008 \text{ d}^{-1}$) were obtained for the experiments performed at the lowest concentration tested (1-1 and 1-2, respectively). Meanwhile increasing that concentration led to significantly higher G_m values.

Conclusions

Methane production from LFHTC via mesophilic anaerobic digestion is a promising approach. The substrate is characterised by high COD and TKN contents, requiring adequate selection of the IC and ISR for a proper operation of the process. High inoculum concentration (25 g COD/L) and/or low ISR (≤ 0.5) affect negatively the ultimate methane yield through methanogenesis inhibition due to the high ammonia nitrogen and VFA released. According to the results, working at an $\text{ISR} \geq 1$ is recommended for the valorisation of the liquid fraction from hydrothermal carbonisation of dehydrated sewage sludge by mesophilic anaerobic digestion.

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References

1. Manara P and Zabanioutou A, Towards sewage sludge based biofuels via thermochemical conversion - A review. *Renew Sust Energ Rev* **16**: 2566-2582 (2012).
2. Danso-Boateng E, Shama G, Wheatley AD, Martin SJ and Holdich RG, Hydrothermal carbonisation of sewage sludge: Effect of process conditions on product characteristics and methane production. *Bioresour Technol* **177**: 318–327 (2015).
3. He C, Gianni, A and Wang JY, Conversion of sewage sludge to clean solid fuel using hydrothermal carbonization: Hydrochar fuel characteristics and combustion behavior. *App Energy* **111**: 257–266 (2013).
4. Kambo HS and Dutta A, A comparative review of biochar and hydrochar in terms of production, physico-chemical properties and applications. *Renew Sust Energ Rev* **45**: 359–378 (2015).
5. Libra JA, Ro KS, Kammann C, Funke A, Berge ND, Neubauer Y, Titirici MM, Fühner C, Bens O, Kern J and Emmerich KH, Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry, processes and applications of wet and dry pyrolysis. *Biofuels* **2**: 89–124 (2011).
6. Sevilla M, Maciá-Agulló JA and Fuertes AB, Hydrothermal carbonization of biomass as a route for the sequestration of CO₂: Chemical and structural properties of the carbonized products. *Biomass Bioenerg* **35**: 3152–3159 (2011).
7. Lynam JG, Reza MT, Yan W, Vásquez VR and Coronella CJ, Hydrothermal carbonization of various lignocellulosic biomass. *Biomass Convers Biorefin* **5**: 173-181 (2015).

8. Broch A, Jena U, Hoekman SK and Langford J, Analysis of solid and aqueous phase products from hydrothermal carbonization of whole and lipid-extracted algae. *Energies* **7**: 62-79 (2014).
9. Stemann J, Putschew A and Ziegler F, Hydrothermal carbonization: Process water characterization and effects of water recirculation. *Bioresour Technol* **143**: 139–146 (2013).
10. Funke A and Ziegler F, Hydrothermal carbonization of biomass: A summary and discussion of chemical mechanisms for process engineering. *Biofuels Bioprod Bioref* **4**: 160–177 (2010).
11. Reza MT, Wirth B, Lüder U and Werner M, Behavior of selected hydrolysed and dehydrated products during hydrothermal carbonization of biomass. *Bioresour Technol* **169**: 352–361 (2014).
12. Berge ND, Ro KS, Mao J, Flora JRV, Chappell MA and Bae S, Hydrothermal carbonization of municipal waste streams. *Environ Sci Technol* **45**: 5696–5703 (2011).
13. Erdogan E, Atila B, Mumme J, Reza MT, Toptas A, Elibol M and Yanik J, Characterization of products from hydrothermal carbonization of orange pomace including anaerobic digestibility of process liquor. *Bioresour Technol* **196**: 35–42 (2015).
14. Weiner B, Wedwitschka H, Poerschmann J and Kopinke FD, Utilization of Organosolv Waste Waters as Liquid Phase for Hydrothermal Carbonization of Chaff. *Sustainable Chem Eng* **4**: 5737–5742 (2016).
15. Wirth B and Mumme J, Anaerobic digestion of waste water from hydrothermal carbonization of corn silage. *App Bioenergy* **1**: 1–10 (2013).

16. Wood BM, Jader LR Schendel FJ, Hahn NJ, Valentas KJ, McNamara PJ, Novak PM and Heilmann SM, Industrial symbiosis: corn ethanol fermentation, hydrothermal carbonization, and anaerobic digestion. *Biotechnol Bioeng* **110**: 2624–2632 (2013).
17. Qiao W, Peng C, Wang W and Zhang Z, Biogas production from supernatant of hydrothermally treated municipal sludge by upflow anaerobic sludge blanket reactor. *Bioresour Technol* **102**: 9904–9911 (2011).
18. Wirth B, Reza T and Mumme J, Influence of digestion temperature and organic loading rate on the continuous anaerobic treatment of process liquor from hydrothermal carbonization of sewage sludge. *Bioresour Technol* **198**: 215–222 (2015).
19. Xiao LP, Shi ZJ, Xu F and Sun RC, Hydrothermal carbonization of lignocellulosic biomass. *Bioresour Technol* **118**: 619–623 (2012).
20. Eibisch N, Helfrich M, Don A, Mikutta R, Kruse A, Ellerbrock R and Flessa H, Properties and degradability of hydrothermal carbonization products. *J Environ Qual* **42**: 1565–1573 (2013).
21. Reza MT, Freitas A, Yang X and Coronella CJ, Wet Air Oxidation of Hydrothermal Carbonization (HTC) Process Liquid. *ACS Sustainable Chem Eng* **4**: 3250–3254 (2016).
22. Ramke HG, Blöhse D, Lehmann HJ and Fettig J, Hydrothermal carbonization of organic waste, in Twelfth International Waste Management and Landfill Symposium, Proc. Sardinia 2009, Italy, ed. Cossu R, Diaz LF and Stegmann R, CISA Publisher (2009).

23. Becker R, Dorgerloh U, Paulke E, Mumm J and Nehls I, Hydrothermal carbonization of biomass: major organic components of the aqueous phase. *Chem Eng Technol* **37**: 511–518 (2014).
24. Smith AM and Ross AB, Production of bio-coal, bio-methane and fertilizer from seaweed via hydrothermal carbonisation. *Algal Res* **16**: 1–11 (2016).
25. Oliveira I, Blöhse D and Ramke HG, Hydrothermal carbonization of agricultural residues. *Bioresour Technol* **142**: 138–146 (2013).
26. Holliger C, Alves M, Andrade D, Angelidaki I et al., Towards a standardization of biomethane potential tests. *Wat Sci Technol* **74**: 2515-2522 (2016).
27. American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater, 20th edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC (1998).
28. Raposo F, de la Rubia MA, Borja R and Alaiz M, Assessment of a modified and optimised method for determining chemical oxygen demand of solid substrates and solutions with high suspended solid content. *Talanta* **76**: 448–453 (2008).
29. Jenkins S, Morgan J and Sawyer C, Measuring anaerobic sludge digestion and growth by a simple alkalimetric titration, *Water Pollut Control Fed* **55**: 448–453 (1983).
30. Hansen KH, Angelidaki I and Ahring BK, Anaerobic digestion of swine manure: inhibition by ammonia. *Water Res* **32**: 5–12 (1998).
31. Rajhi H, Puyol D, Martínez MC, Díaz EE and Sanz JL, Vacuum promotes metabolic shifts and increases biogenic hydrogen production in dark fermentation systems. *Front Environ Sci Eng* **10**: 513–521 (2016).

32. Rozzi A and Remigi E, Methods of assessing microbial activity and inhibition under anaerobic conditions: a literature review. *Rev Environ Sci Biotechnol* **3**: 93–115 (2004).
33. Molina F, Ruiz-Filippi G, Garcia C, Lema JM and Roca E, Pilot-scale validation of a new sensor for on-line analysis of volatile fatty acids and alkalinity in anaerobic wastewater treatment plants. *Environ Eng Sci* **26**: 641-649 (2009).
34. Raposo F, De la Rubia MA, Fernandez-Cegri V and Borja R, Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures. *Renew Sust Energ Rev* **16**: 861–877 (2011).
35. Garcia C, Molina F, Roca E and Lema JM, Fuzzy-based control of an anaerobic reactor treating wastewaters containing ethanol and carbohydrates. *Ind Eng Chem Res* **46**: 6707-6715 (2007).
36. Silvestre G, Fernández B and Bonmatí A, Addition of crude glycerine as strategy to balance the C/N ratio on sewage sludge thermophilic and mesophilic anaerobic co-digestion. *Bioresour Technol* **193**: 377–385 (2015).
37. Nielsen HB, Uellendahl H and Ahring BK, Regulation and optimization of the biogas process: Propionate as a key parameter. *Biomass Bioenerg* **31**: 820–830 (2007).
38. Lahav O and Morgan BE, Titration methodologies for monitoring of anaerobic digestion in developing countries—a review. *J Chem Technol Biotechnol* **79**: 1331–1341 (2004).
39. De la Rubia MA, Walker M, Heaven S, Banks CJ and Borja R, Preliminary trials of in situ ammonia stripping from source segregated domestic food waste digestate using biogas: Effect of temperature and flow rate. *Bioresour Technol* **101**: 9486–9492 (2010).

40. Niu Q, Qiao W, Qiang H, Hojo T and Li YY, Mesophilic methane fermentation of chicken manure at a wide range of ammonia concentration: stability, inhibition and recovery. *Bioresour Technol* **137**: 358–367 (2013).
41. Chen Y, Cheng JJ and Creamer KS, Inhibition of anaerobic digestion process: a review. *Bioresour Technol* **99**: 4044–4064 (2008).
42. Pellerá FM and Gidarakos E, Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste. *J Environ Chem Eng* **4**: 3217–3229 (2016).
43. Taherzadeh MJ and Karimi K, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *Int J Mol Sci* **9**: 1621-1651 (2008).
44. Rodríguez-Chiang L.M and Dahl OP, Effect of inoculum to substrate ratio on the methane potential of microcrystalline cellulose production wastewater. *Bioresour* **10**: 898-911 (2015).
45. Yoon YM, Kim SH, Shin KS and Kim CH, Effects of substrate to inoculum ratio on the biochemical methane potential of piggery slaughterhouse wastes. *Asian Australas J Anim Sci* **27**: 600-607 (2014).
46. Hashimoto AG, Pretreatment of wheat straw for fermentation to methane. *Biotechnol Bioeng* **28**: 1857–1866 (1986).

Table 1. Experimental conditions and notation of the anaerobic digestion experiments

Inoculum		Substrate			
ISR on a COD basis		2	1	0.5	0.4
ISR on a VS basis		2.6	1.3	0.6	0.5
Concentration (g COD/L)	10	5.0	10.0	20.0	25.0
NOTATION in the text		1-2	1-1	1-0.5	1-0.4
Concentration (g COD/L)	25	12.5	25.0	50.0	62.5
NOTATION in the text		2.5-2	2.5-1	2.5-0.5	2.5-0.4

Table 2. Values of pH, alkalinity and intermediate to total alkalinity ratio from the anaerobic digestion experiments

Experiment	pH		Alkalinity (g CaCO ₃ /L)		IA/TA	
	Initial	Final	Initial	Final	Initial	Final
1-2	7.5	7.7	1.02	2.19	0.32	0.26
1-1	7.3	7.9	1.46	3.78	0.51	0.21
1-0.5	6.5	7.0	1.81	4.10	0.80	0.76
1-0.4	6.1	6.9	1.98	4.56	0.88	0.80
2.5-2	7.6	8.0	2.31	5.36	0.38	0.29
2.5-1	7.0	7.5	2.85	5.95	0.67	0.70
2.5-0.5	5.5	7.4	2.78	7.27	1.00	0.79
2.5-0.4	5.4	7.5	4.33	9.49	0.83	0.78

Table 3. Compounds identified by GC/MS in the liquid fraction from hydrothermal carbonisation of sewage sludge and removal efficiencies upon anaerobic digestion

Compound	Removal efficiency (%) [*]		
	2.5-2	1-2	1-1
<i>Aldehydes</i>			
4-Methoxycinnamaldehyde	> 99	> 99	> 99
2,3,6-Trichlorobenzaldehyde	nd	gen	gen
<i>Nitrogenates compounds</i>			
4-Pentyloxyaniline	> 99	> 99	> 99
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	0	> 99	0
Pyrazine, 2-ethyl-5-methyl-	0	0	0
Benzenamine, 3-methoxy-	0	0	9
4,5-Dimethyl-ortho-phenylenediamine	0	0	34
2,3-Diethylpyrazine	0	0	13
Pyrazine, 2,5-dimethyl-3-propyl-	15	0	45
2(1H)-Quinoxalinone	> 99	> 99	> 99
1-Butanamine	> 99	> 99	> 99
1H-Indole, 7-methyl-	gen	nd	nd
<i>Oxygenated aromatics</i>			
Furan, 2,5-dimethyl-	> 99	95	> 99
Benzene, 1,2,4,5-tetramethyl-	0	0	22
Phenol, 2,3,5,6-tetramethyl-	25	13	35
Benzoic acid, 4-formyl-	20	10	30
Phenol, 2-methyl-6-(2-propenyl)-	12	> 99	26
Phenol, 2-methyl-5-(1-methylethyl)-	> 99	> 99	> 99
Benzophenone	> 99	> 99	> 99
Phenol, 2,4-bis(1,1-dimethylethyl)-	gen	gen	gen

^{*}with respect to peak area

nd: not detected

gen: generated

Table 4. Values of the apparent kinetic constant (k) and maximum methane yield (G_m)

Experiment	G_m (mLCH ₄ /g COD _{added})	k (d ⁻¹)	R^2
1-2	166±17	0.043±0.008	0.966
1-1	168±14	0.048±0.008	0.972
2.5-2	237±18	0.031±0.003	0.978

FIGURE CAPTIONS

Figure 1. Time-course of total VFA at different inoculum concentration and ISR values

Figure 2. Initial TKN values (tables) and time-course of total ammonia nitrogen at different inoculum concentration and ISR values

Figure 3. Time-course of soluble COD at different inoculum concentration and ISR values

Figure 4. Cumulative methane yield (symbols) at different inoculum concentration and ISR values. The lines show the fittings to the rate equation (1)

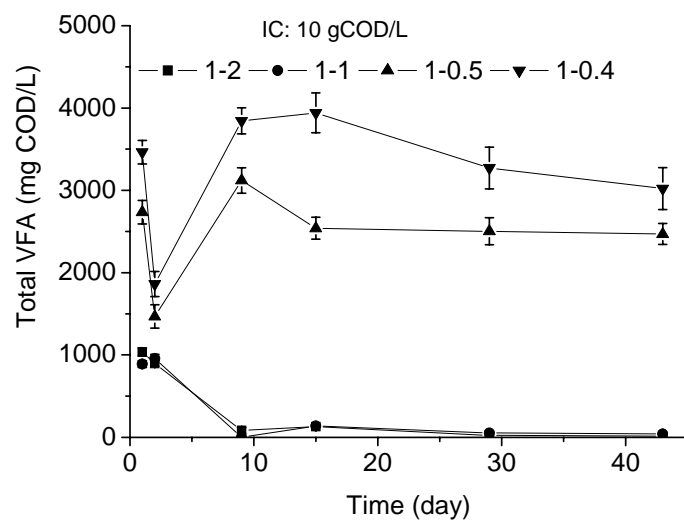
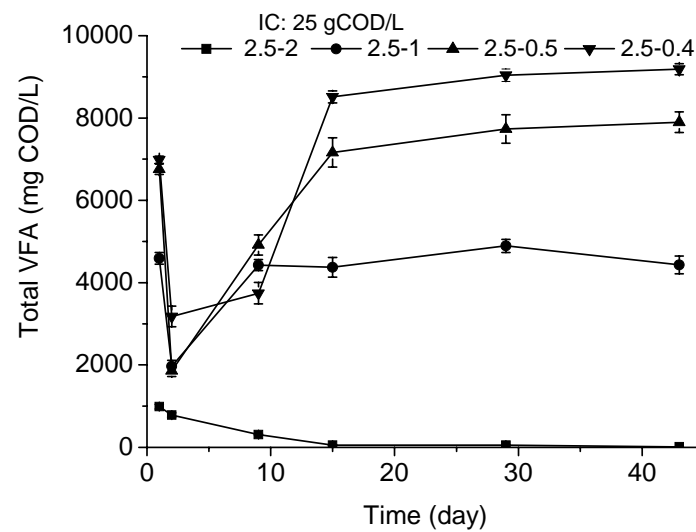


Figure 1.



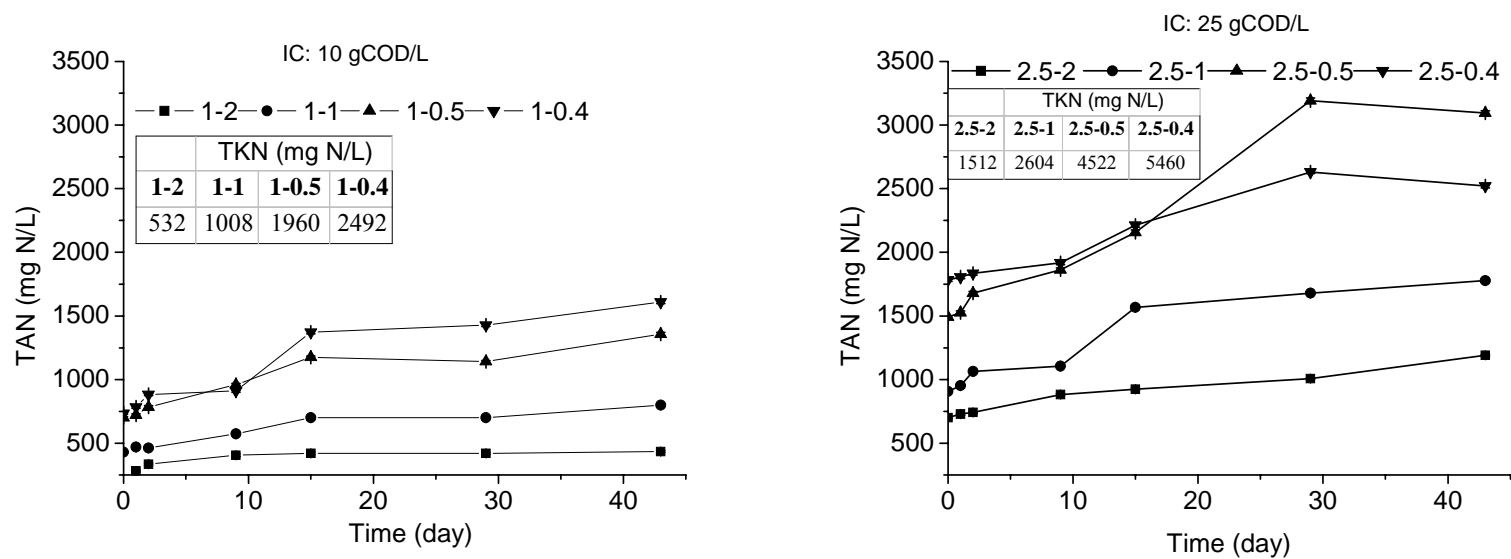


Figure 2.

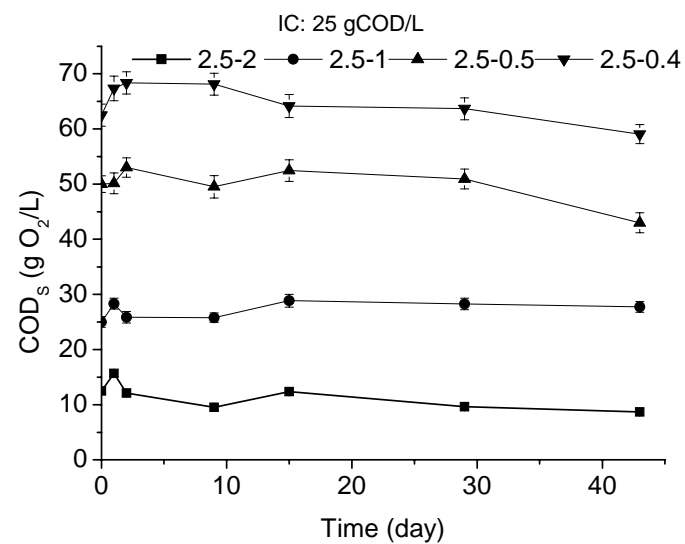
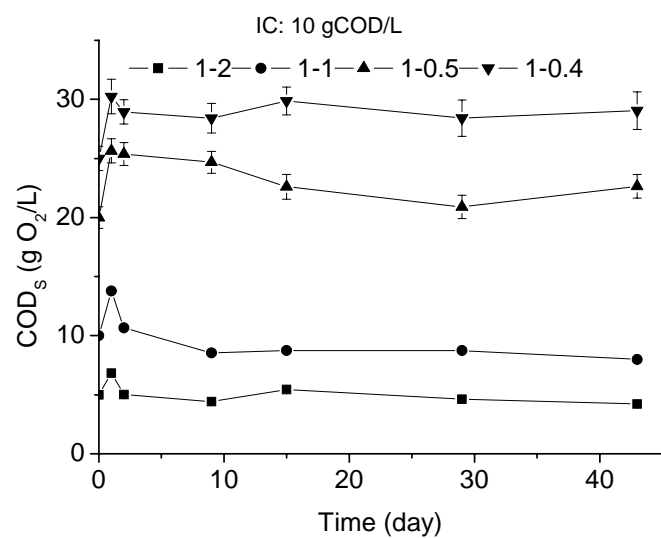


Figure 3.

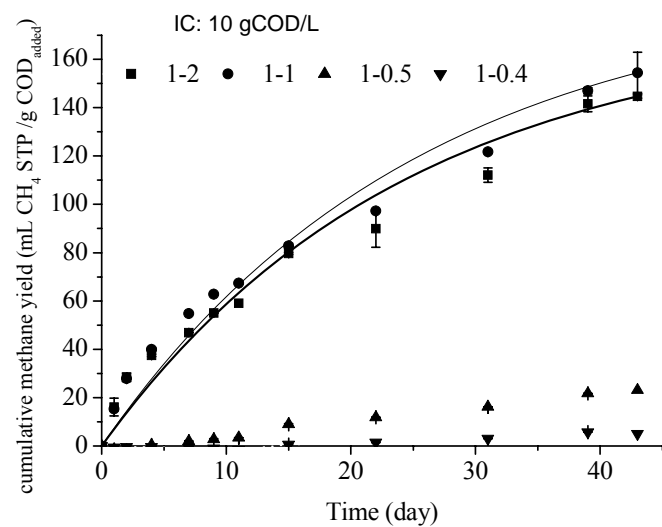


Figure 4.

