Co-digestion of organic and mineral wastes for enhanced biogas production: reactor performance and evolution of microbial community and function

Burhan Shamurad, Neil Gray[1], Evangelos Petropoulos[1], Shamas Tabraiz[1], Kishor Acharya[1], Marcos Quintela-Baluja[1] and Paul Sallis[1]

[1]School of Engineering, University of Newcastle-upon-Tyne, UK

Corresponding author:

Burhan Shamurad**,** PhD researcher in Environmental Engineering, School of Engineering, Room G20, Cassie Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK.

Email: b.a.s.shamurad@ncl.ac.uk

Abstract

Mineral wastes (MWs) from municipal solid waste incineration plants and construction demolition sites are rich in minerals, heavy metals and have acid neutralising capacity. This renders such MWs a promising source of bulk and trace elements to enhance and stabilize biogas production in anaerobic processes. However, finding a MW with typical heavy metal concentrations, which promotes anaerobic digestion (AD) without adverse effects on the microbial community of the reactor is of major importance. To investigate the impact of several MW additives (1. incineration bottom ash; 2. fly ash; 3. boiler ash; 4. cement-based waste) as AD co-substrates, six 5 L single stage mesophilic, continuously stirred tank reactors (CSTR) were setup. Two different feeding regimes were employed including: a) a liquid-recycled feeding method (LRFM); b) a draw-and-fill feeding method (DFFM). Under the LRFM regime, one gram MW per gram organic waste enhanced process stability (pH), increased methane production (25 - 45% increase), and yielded (450 – 520 mL CH_4/g VS); DFFM enhanced digestibility to a lesser degree. Illumina HiSeq 16S rRNA community sequencing of reactors showed that the microbial community compositions were unaffected by the presence of MW additives in comparison to unamended controls, but MW amendment accelerated bacterial growth (determined by qPCR). In contrast, different feeding regimes altered the microbial communities; *Methanoculleus* (hydrogenotrophic) and *Methanosaeta* (acetoclastic) were the most

abundant methanogenic genera in the LRFM reactors, and the more metabolically versatile *Methanosarcina* genus dominated under DFFM.

Keywords: Co-digestion, Mineral wastes, Methanogenic activity, Microbial diversity, Microbial population, Process stability

1. Introduction

The potential reuse of the incineration ash can effectively lower disposal costs of MSW and provide valuable materials to countries where natural resources are either expensive and/or unavailable (Liu *et al.*, 2015). In 2016, the amount of solid waste incinerated in the EU-28 reached 68 million tonnes (i.e. a 105% increase since 1995) (eurostat, 2018). In the UK, incinerated solid wastes accounted for 7.6 million tonnes in 2014 (DEFRA, 2018). Moreover, each year 2.4 Gt of Portland cement is manufactured and used by the construction industry worldwide (Renforth *et al.*, 2011). Both these activities produce mineral wastes (MW), namely, ashes and demolition materials only a minor fraction of which is re-used by the construction industry with most either disposed or used as cover layers in landfills (Chandler *et al.*, 1997).

Such solid mineral wastes (MWs) discharged from municipal solid waste incineration (MSWI) plants (incineration bottom ash (IBA), fly ash (FA) and boiler ash (BA)), and construction demolition waste (CDW), referred to as cement-based waste (CBW), are rich in minerals which have an acid neutralising capacity and have associated

heavy metals (Chimenos *et al.*, 1999; Washbourne *et al.*, 2015). The presence of metals and pH buffering capacity are properties that render such MWs a resource that may have a promising impact in anaerobic digestion (AD) processes where these properties are key process regulation parameters. However, in AD, the heavy metals present in such MWs might be stimulatory, inhibitory or toxic depending upon their concentration (Hickey *et al.*, 1989; Lin, 1993b; Mudhoo and Kumar, 2013; Franke-Whittle *et al.*, 2014). From a stimulatory perspective, microorganisms require certain trace concentrations of metals for activation and/or function of enzymes and co-enzymes (Zandvoort *et al.*, 2006; Abdel-Shafy and Mansour, 2014). However, metal concentration above certain thresholds may cause inhibition co-depending on other physicochemical characteristics in AD digesters (VS/TS, humic substances, pH, VFA, alkalinity and ammonia (Dong *et al.*, 2013)). The key parameter controlling the potential toxicity of metals is the concentration of the solids (VS or TS) (Hickey *et al.*, 1989; Mudhoo and Kumar, 2013). Moreover, (Gu and Wong, 2004) identified impacts of VFA concentration (acetic and propionic acid) on metal solubilisation during bioleaching of sewage sludge. This study found that the presence of 10.8 mM acetic acid and 9.88 mM propionic acid delayed solubilisation of Cu and Cr by 6 and 7 days respectively compared to a one day lag period in the control with low organic acid concentration.

Anaerobic co-digestion of organic wastes with CBW has not been found in the literature. However, incineration bottom ash (IBA) from MSWI plant solid residues has

gained some attention in the context of the AD of OFMSW (Banks and Lo, 2003; Lo, 2005). The aim of the current study was to investigate the impact of integrating different types of MWs during the AD of the organic fraction of MSW. The hypothesis was that the release of alkalinity, necessary macronutrients (Ca, Na, K and Mg) and trace metals (Fe, Zn, Mn, B, Co, Ni, Cu, Mo, Se, Al, W and V) would benefit the process and promote digestibility as assessed by biogas production and stability with additional insights into mechanistic effects of additives provided by microbial community abundances and dynamics.

This current study of co-digestion of mineral and organic wastes was conducted using a liquid-recycled feeding method (LRFM) based on the hypothesis that recycling the liquor part of digestate could affect AD through retaining metals considered either stimulatory or inhibitory (toxic) to the digestion processes (Gu and Wong, 2004; Mudhoo and Kumar, 2013). In addition, for the purpose of comparison, one of the reactors was fed using the conventional draw-and-fill feeding method (DFFM). With this feeding regime the substrate was IBA co-digested with organic wastes.

2. Materials and methods

2.1 Inoculum and Substrate

The AD inoculum was obtained from a mesophilic (37^{o}C) AD plant treating cattle slurry and food waste (Cockle Park Farm, Newcastle University, UK). The inoculum

(seed) was stored at -20^{o}C until use, then activated for 7 days (VDI, 2006) at 37^{o}C before the substrate acclimation period. The substrate used for AD was a synthetic organic waste (SOW) composed of 79% cooked food leftovers (such as rice 13.6%, meat 1.5%, beans 5.6% and fat 1.4%), 20% of uncooked fruit and vegetable wastes (such as apple 1.3%, orange 1.7%, banana 2%, lemon 1.2% and pomegranate 1.4% and herbs ~ 6%), and 1% of packing cardboard, to simulate the composition of an organic matter going to landfill.

Representative study by Forster-Carneiro *et al.* (2008) suggested that a total solid of 10 - 20% is capable of producing an inert bio-solid product with high methane productivity. Therefore, the SOW total (TS) and volatile (VS) solids concentrations were adjusted to 18.7% and 16.6% by wet weight respectively using distilled water (a 30% volume dilution of the blended organic waste was required). After adjustment, the substrate was stored at -20^{o}C until use. The suitability of the substrate (synthetic organic waste (SOW)) for AD was assessed by measuring the bio-methane potential (BMP) of the substrate according to the VDI method (VDI, 2006) and the substrate (SOW) theoretical methane yield was calculated using the elemental composition analysis according to the equation described in (Nielfa *et al.*, 2015). The physicochemical characterization of the inoculum and SOW are shown in Table 1 and Table 2.

2.2 Mineral waste additives

Three co-substrate MWs (IBA, FA and BA) were obtained from a domestic Waste-to-Energy incineration power plant, Teesside, UK. In this plant, household food and garden wastes are dried and burned at ≈ 1000^{o}C; steam turbines then convert the produced heat to energy. An additional MW residue of CDW was produced from two CBW samples (with nominal particle sizes of 10 mm and 1 mm) collected from a CDW recycling site in Newcastle upon Tyne, UK. All the MW samples (IBA, FA, BA, and CBW) were dried overnight (104^{o}C) and visible metals, glass and plastic materials removed. Prior to being added to the feed, all MWs were ground by a mill (Vibratory Disc Mills, SIEBTECHNIK-TS, Germany), sieved to less than 212 µm (BS410 standard sieves 212 µm diameter) and stored at room temperature in airtight containers until use. The physicochemical characterization of the MWs are shown in Table 2.

Reactors: six anaerobic, lab-scale completely stirred-tank reactors (CSTR) were setup. Each of the six reactors (5 L working volume) was a borosilicate glass, flat bottomed, cylindrical sided, flask with three glass quick fit (100 mm bore) openings. The reactors, identified as LFA, LCO, DIBA, LCBW, LBA and LIBA, respectively, reflecting their respective feeds (Table 3) operated at mesophilic temperature (37^{o}C) with HRT/SRT of 20 days (Viswanath *et al.*, 1992; El-Mashad and Zhang, 2010). Initially, each of the six reactors was thoroughly filled (5 L) with inoculum to get acclimated to the substrate and environment for 20 days. During acclimation, the reactors were fed every 2 - 3 days with a

129 SOW and distilled water mixture containing 1 g SOW volatile solids and the biogas
130 produced by the CSTR systems were collected in gasbags and checked for methane
131 volume and composition to ensure the microorganisms were active. On day 20 a methane
132 content of > 50% was observed in all the reactors indicating that they were ready for
133 operation in the codigestion experiments.

134 During the codigestion experiments, the reactors were run for 75 days to ensure pseudo-
135 steady state conditions had been achieved (> 3 HRT, (Dai *et al.*, 2013)) with two organic
136 loading rates (OLRs): 0.5 g VS/L.d for days 0 - 40 and 1 g VS/L.d for days 41 - 75
137 successively with or without additions of MW to give $MW_{(TS)}/SOW_{(VS)}$ at a mass ratio of 1
138 to 1.

139 **Feeding:** the current study of the co-digestion of mineral and organic wastes was
140 conducted under a reactor-feeding method named as 'liquid-recycled feeding method' or
141 LRFM. This is based on the hypothesis that recycling the liquid fraction of the digestate
142 could reduce losses of metals that are considered either stimulatory or inhibitory (toxic) to
143 the digestion processes (Gu and Wong, 2004; Mudhoo and Kumar, 2013) by returning
144 them to the reactor in each feeding cycle. For comparison, the two feeding methods,
145 conventional draw-and-fill feeding method (DFFM) and the LRFM were used in the
146 reactor fed with SOW feed and supplemented with IBA to determine the effect of feeding
147 regime on MW supplementation in AD of organic waste (SOW).

148 The liquid-recycled feeding method (LRFM) was applied for the LFA, LCO, LCBW, LBA
149 and LIBA reactors (the first letter of the reactor name refers to the feeding method used
150 whereas the following letters refers to individual MW additions). For this feeding method,
151 instead of using distilled water for preparing the required volume of the daily feed (a 250
152 ml mixture), the liquid fraction of the discharged digestate from each reactor (sieved with a
153 212 µ sieve) was used (the solid fraction of the digestate was discarded). The draw-and-fill
154 feeding method (DFFM) was applied for the DIBA reactor whereby the volume was
155 maintained at 5 L by withdrawing digestate and feeding equal volumes of feed prepared
156 with DW instead of the liquid fraction of the digestate. The experimental design of the
157 reactor systems is shown in Table 3.

158 **2.3 Analytical methods**

159 Five-litre gasbags (Tedlar, VWR) were used for biogas collection from the reactors.
160 Each day, the gasbags were disconnected, biogas volume measured, emptied with samples
161 of biogas from each analysed for methane and carbon dioxide composition by gas
162 chromatography then the gasbags were reconnected to the reactors. Triplicate samples of
163 biogas, each of 50 µl, were taken from the gasbags using a lock tight gas syringe (SGE,
164 Australia) and analysed for methane and carbon dioxide by gas chromatography (Carlo
165 Erba HRGC S160 GC with MFC 500 detector). The GC was equipped with an Agilent HP-
166 PLOTQ column (0.32 mm diameter, 30 m length and 20 µm film, Agilent, UK). The GC
167 carrier gas was hydrogen (250 mL/min) with an oven temperature held isothermally at

35°C. Digestate pH was measured using a pH meter (Jenway, 3310). For the SOW and MWs, one volume of each sample dissolved in two volume of distilled water mixed with magnetic stirrer for one hour then measured for pH. Samples of digestate were also taken on a weekly basis and centrifuged (3392 x g, 30 min) and the supernatant was used for determining: chemical oxygen demand (COD); ammonia-nitrogen (NH_3-N) and; total Kjeldahl nitrogen (TKN). Contamination of the reactor headspace with lab air was prevented during the feeding and sampling processes because the sampling port extended below the liquid surface level of the reactor. Analyses were determined according to the standard methods for the examination of water and wastewater (AWWA, 2012). Total volatile fatty acids (TVFA) and total alkalinity (TALK) were measured by titration according to the Lossie and Pütz (2008) method. Total solids (TS) were calculated as the mass of solids remaining after oven- drying digestate samples overnight (105^{o}C); volatile solids (VS) mass was calculated after oven drying at 550^{o}C for 30 minutes.

Metal analysis for the raw SOW and MWs, and digestate samples (on day 75) were performed according to the EPA method 3010A (see section 2.1 in (Shamurad *et al.*, "in press")) using an ion coupled plasma optical emission spectrometer (Vista MPX simultaneous ICP - OES). An elemental assay for the percentage of C, N, and S in the dried digestate samples was performed using an organic element analyser (Elementar Vario MAX CNS) according to the manufacturer's instructions and the standard method (SCA, 1986) . An element assay was carried out at an external lab (Elemental

Microanalysis Ltd, UK) to measure the C, N, S, H and O elements of the SOW. To explore possible stimulatory mechanisms various physiochemical parameters including the concentrations of metal elements in the digestate together with the reactor performances on day 75 were subjected to correlation analysis using the IBM SPSS Statistics version 23 (IBM, 2015).

2.4 Molecular analysis

Microbial community analyses were performed for a sample of inoculum on day 0 (before the acclimation period) and in digestate samples collected on day 20 and day 75. Genomic DNA was extracted according to the modified protocol of Griffiths *et al.* (Griffiths *et al.*, 2000). The quality and concentration of extracted DNA was measured with a Nano drop spectrophotometer (Thermo Fisher, UK), (see section 2.2 in (Shamurad *et al.*, "in press")).

Real time quantitative PCR (qPCR): Real time quantitative PCR assays were conducted both on the inoculum and on the digestate DNA extracts. Targeting the *mcrA* gene (mlas-F and mcrA-R primers) as a measure of the abundance of methanogens (Steinberg and Regan, 2009) and targeting the 16S rRNA gene (1055F and 1392R primers) as a measure of total bacteria (Harms *et al.*, 2003). Real-time qPCR assays were performed using a BioRad CFX C1000 System (BioRad, Hercules, CA USA); (see section 2.2 in (Shamurad *et al.*, "in press")).

16S rRNA gene sequencing: an Illumina Hiseq (16S V4) library by (Earlham Institute, UK) was prepared after the DNA extracts were quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Q33231) and sample purity was checked on the DropSense 96 (Perkin Elmer); (see section 2.2 in (Shamurad *et al.*, "in press")).

Sequenced data processing and statistical analysis: raw sequencing data (FastQ files) obtained from the Illumina sequencing platform were de-multiplexed and quality filtered using dada2 (Callahan *et al.*, 2016) within the QIIME2 analysis pipeline (https://qiime2.org, (Caporaso *et al.*, 2010). Closed-reference operational taxonomic unit (OTU) picking was performed using VSEARCH (Rognes *et al.*, 2016) using the 'cluster-features-closed-reference' plugin in QIIME2, using the SILVA119 reference database to produce a table detailing the frequencies of taxonomically assigned representative sequences within individual sample libraries (see section 2.2 in (Shamurad *et al.*, "in press")). Further analysis was conducted on these data to generate figures and check microbial diversity using the phyloseq (McMurdie and Holmes, 2013) and STAMP v2 (Parks *et al.*, 2014) software packages.

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 7.0 (Kumar *et al.*, 2016). Evolutionary histories were inferred using the Neighbour-Joining method (Saitou and Nei, 1987). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2011) and the percentage of

replicate trees in which the associated taxa clustered together was determined by bootstrap analysis of 1000 replicates (Westerlund and Edgerton, 2007).

***Microbial specific activity*:** The cell specific methanogenic and fermentation activities on day 75 was estimated from the daily methane production and total COD concentration (total COD concentration equals to the sum of the soluble COD (sCOD) concentration in the digestate plus the methane production expressed as COD). The average number of the methanogenic and bacterial populations on day 75 was estimated from the qPCR analysis. For total bacteria enumeration, the 16S RNA gene abundances were divided by 4 and for total methanogens the mcrA gene abundances were divided by 2 (Klappenbach *et al.*, 2001). The formulas (Equations 1 and 2) provided by Petropoulos *et al.* (2017) were employed for the estimation of the two specific activities:

$$Cell\ specific\ methanogenesis = \frac{Methane\ production\ (ml)}{Number\ of\ methanogen\ cells} \quad \text{... Equation 1}$$

$$Cell\ specific\ hydrolysis = \frac{Total\ COD\ concentration}{Number\ of\ bacteria\ cells} \quad \text{.... Equation 2}$$

3. Results and discussion

3.1 Performance characteristics of the AD reactors

The experimental BMP value of the SOW was 480 ± 50 mL CH_4/g VS. This value was close to the calculated (theoretical) value (514 mL CH_4/g VS) estimated from the elemental composition analysis according to the method described in (Nielfa *et al.*, 2015)

245 and was within the typical range of BMP values reported for food wastes ((435 - 489 mL
246 CH_4/g VS); (Zhu *et al.*, 2008; Banks *et al.*, 2011; Nielfa *et al.*, 2015)). This outcome
247 indicates that the substrate used in this study was suitable for the digestion studies
248 conducted.

249 Figure 1 and Table 4 show the performance profiles and physicochemical parameters
250 of the six reactors operated in this study. On day 20, in the six reactors, the average
251 methane yield was 499 ± 38 mL CH_4/g VS, with close to equal values of pH (6.9 ± 0.16)
252 and NH_3-N (533 ± 49 mg/L), indicating similar and stable reactor conditions. According to
253 (Koster and Lettinga, 1984; Ward *et al.*, 2008; Franke-Whittle *et al.*, 2014) AD occurs
254 optimally at pH values of 6.8 - 7.2 and total ammonia nitrogen concentrations below 1700
255 mg/L. Presumably, the inoculum used in the CSTR set-ups contained enough alkalinity (to
256 balance pH) and nutrients (Table 1) for the digestion processes to be stable until day 20,
257 therefore between days 0 - 20 all the reactors showed approximately similar digestion
258 conditions. Thereafter, and with continuous daily feeding and gradual dilution of the set-up
259 inoculum and substrate inside the reactors, the parameters inside the reactors represented
260 the conditions induced by the daily feeds which now diverged (SOW or SOW and MW).

261 In the LRFM, the contribution of the MWs in the LBA, LCBW and LIBA reactors
262 toward the alkalinity balance was detectable; these reactors showed 1000 - 1500 mg/L
263 more alkalinity than that in the control reactor, however, in the DIBA reactor (fed with
264 DFFM) the buffering capacity of the IBA was limited with a noticeable decrease in the

alkalinity. A similar decrease in the alkalinity was observed in the control (LCO) reactor, specifically from day 40 and onwards when organic loading rates were increased from 0.5 g VS/L.d to 1.0 g VS/L.d. The alkalinity in the LCO and DIBA reactors declined from an average concentrations of 2500 mg/L on day 40 to about 1000 - 15000 mg/L by day 75 (Figure 1c), resulting in a pH drop from 6.9 ± 0.1 on day 40 to ~ pH 5.8 on day 75. Among the reactors amended with the MWs and fed with LRFM only the LFA reactor showed a lower alkalinity (~ 1250 mg/L) with a pH (6.4) on day 75.

Under the LRFM feeding regimen, co-digestion of the SOW and mineral wastes (IBA, FA, BA and CBW) resulted in higher methane yields and stable digestion process compared with the control (LCO) (Figure 1 and Table 4). The highest daily methane production was from LCBW, LIBA, LBA and LFA reactors with 528, 513, 468 and 446 mL/L.d respectively. Daily methane production in the DIBA reactor (operated with DFFM) was 376 mL/L.d, which was about 30% lower than the mean daily methane production of the reactors operated with LRFM. Correspondingly, on day 75, the accumulated methane volume produced by the LFA, LCBW, LBA and LIBA reactors were 27, 45, 28 and 44% higher than the LCO (control) reactor, respectively. For the DFFM, the accumulated methane volume of the DIBA reactor (amended with IBA) was 24% higher than the control but about 25% lower than that of the LCBW and LIBA reactors.

3.2 The influence of reactor amendments and feeding regimens on microbial abundances

The overall performance of the reactors i.e. higher stable biogas production and stable pH and VFA levels with MW supplements compared to when MW is absent suggests that these materials primarily promote the growth and survival of the microbes present in the reactors increasing the volumetric rate of hydrolysis and fermentation. This growth leads to the increased formation of intermediate substrates for methanogens but equivalent increase in consumption of these products by the methanogens (Hude Moreshwar and Yadav Ganapati, 2014) (following a Monod and Michaelis-Menten approach respectively). To test this hypothesis, microbial abundances were determined in these reactor systems and cell specific activities calculated.

Methanogenic populations: the *mcr*A gene abundances representing methanogenic populations in the control and MW amended reactors showed an increase in numbers between days 0 to day 75. On day 75, the methanogen abundances in the control and MW amended reactors were 80 (4.44×10^9 genes/mL) and 90 - 118 - fold (4.94×10^9 - 6.50×10^9 genes/mL) higher than the inoculum (5.52×10^7 genes/mL) on day 0 respectively (Figure 2). In the LFA and DIBA reactors, the methanogen abundances were lower than in the control by day 75. Methanogenic populations in the DIBA reactor by day 75 (1.08×10^8 genes/mL) had only increased two-fold compared to the inoculum was the lowest population size observed among all the reactors (Figure 2).

Bacterial populations: on day 75, the 16S gene abundances representing bacterial populations in the LCBW and LIBA reactors were 11- and 14-fold (1.4×10^{12} and 1.8×10^{12} genes/mL) higher respectively than that in the inoculum (1.2×10^{11} genes/mL) on day 0. While in the LCO (7.2×10^{11} genes/mL), LBA (5.8×10^{11} genes/mL) and LFA (7.3×10^{11} genes/mL) reactors the 16S gene abundances were 4 - 5-fold higher than that in the inoculum on day 0. The lowest 16S gene abundance increase was in the DIBA reactor (2.7×10^{11} genes/mL) which was only one-fold higher that than in the inoculum on day 0 (Figure 2). There was no notable difference in the bacterial population between the LFA reactor (7.3×10^{11} genes/mL) and the control (LCO) reactor on day 75; however, bacterial gene abundances in the LCBW and LIBA reactors were about 2.0 - 2.6-fold higher respectively than the control.

The cell specific hydrolysis and methanogenesis activities for the reactors were estimated from measured CODs, biogas production and the relative abundances of the bacteria and methanogens from the qPCR analysis on day 75 (section 2.4 and Table 4). In the LRFM reactors, the LFA reactor showed the highest cell specific methanogenic and hydrolytic activities at 0.049 pmol CH_4/cell.d and 0.081 pgram COD/cell.d, respectively. That is to say in this reactor, which actually sustained the lowest LRFM with MW biogas production, the growth/maintenance of individual cells apparently required higher rates of substrate turnover especially with respect to the methanogen population. The control reactor (LCO) had a moderately high cell specific hydrolysis activity of 0.069 pgram

COD/cell.d and relatively similar methanogenic activity (0.015 pmol CH_4/cell.d) compared to reactors amended with MWs amended reactors operated with LRFM (except for LFA). The LIBA reactor had cell specific hydrolytic and methanogenic activities of 0.028 pgram COD/cell.d and 0.024 pmol CH_4/cell.d respectively. Whilst, the DIBA reactor (which had the same MW added as the LIBA reactor) showed the highest cell specific hydrolytic and methanogenic activities (0.106 pgram COD/cell.d and 0.363 pmol CH_4/cell.d respectively).

In the AD, hydrolysis and the primary and secondary fermentation process are mainly linked to bacteria (Liebetrau *et al.*, 2017). However, in methanogenic syntrophic partnerships it is well understood that the activity of the fermentative bacteria can be limited by the inhibition (failure) of the methanogens, because such inhibition results in the accumulation of both sCOD and total VFAs (as summation of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate) in the reactor which will eventually stop fermentation (Berlanga Herranz, 2008). It is also well understood that the success of such syntrophic partnerships is principally controlled by thermodynamic trade-offs between the partners and the efficient transfer of substrate intermediates between them whereby the energy yield for each participant in the partnership is maximal (Hamilton *et al.*, 2015). The accumulation of VFA as a control of bacterial hydrolysis and fermentation was certainly evident in the control reactor (LCO) on day 75 (~ 4 g/L) (Figure 1d). Suggesting that the methanogen population was limiting VFA conversion to methane, while in the reactors amended with the MWs (LFA, LCBW, LBA and LIBA) and operated with LRFM the

343 VFA concentration on day 75 remained less than 0.5 g/L. Presumably, the MW in
344 particular stimulated methanogenesis (as is obvious in LFA), which in turn stimulated
345 bacterial growth (as it is obvious in LBA and LCBW) whereby the removal of the
346 fermentation products increased the energy yield for fermentation (Berlanga Herranz,
347 2008).

348 Optimising reactor performance by additions of MW may allow not only the
349 reduction of the applied HRT, but also a capital and maintenance cost minimization as a
350 direct consequence. Since biodegradation is an intrinsic property linked to biomass growth
351 kinetics, an increase of the cell concentration within a reactor, combined with an increase
352 in the activity of each cell gives process intensification, and the possibility of using smaller
353 reactors (Akay *et al.*, 2005). Importantly, the readily available source of the MW
354 supplements, combined with its minimal processing requirement, makes it a promising
355 material for AD optimization in regions where commercial trace element additive solutions
356 are either expensive and/or unavailable. Moreover, MW supplementation may have
357 benefits in other sectors, for example where hydrolysis/fermentation is carried out at low
358 temperatures and metabolic reaction rates are consequently reduced (Petropoulos *et al.*,
359 2017). Furthermore, the use of MWs from MSWI plants in AD decrease the amount of
360 MWs need to be sent to landfills as a daily cover material (Banks and Lo, 2003). The use
361 of MWs as a daily cover material of landfill will enhance biological degradation of wastes
362 and increase the landfill capacity to receive an increasing quantity of the daily wastes.

Indeed, the landfills with a daily cover of mineral wastes could themselves be converted to an AD bioreactor for biogas production (the leachate produced from the landfill can be recycled again to the landfill like the LRFM applied in this study) giving an economic value to the MWs.

3.3 The contributions of metals from MWs to AD digestates and correlation of physiochemical parameters with reactor performances

Additions of trace elements like Ni, Co, Fe, Mn, Zn Mo etc. either singly or, in combination, to anaerobic reactors are known to be sometimes necessary for the activity of the enzymes improving methanogenesis (Oleszkiewicz and Sharma, 1990; Feng *et al.*, 2010; Pobeheim *et al.*, 2010; Takashima *et al.*, 2011; Zhang *et al.*, 2011; Zhang *et al.*, 2012; Facchin *et al.*, 2013; Westerholm *et al.*, 2015; Westerholm *et al.*, 2016; Wu *et al.*, 2016). Accordingly, the positive effects of MW amendments on the microbial populations involved in the reactors described above may be due to the increased supply of such required nutrients in addition to, or, alternative to, the provision of alkalinity to keep pH values within optimum range. Certainly, the analysis of metals in the MWs revealed they are orders to many orders of magnitude richer in most major, minor and trace metals when compared to the SOW substrate. This richness is reflected in the compositions of the digestate solids (see Figure 2 and 3 in (Shamurad *et al.*, "in press")), whereby the unamend control has lower levels of most metals in comparison to the mixtures. Furthermore, this variation and general trend is consistently reflected, particularly in the minor and trace

383 metals, in increased concentrations of B, Ba, Cd, Co, Cr, Mn, Mo, Ni, Pb in the LRFM
384 reactors.

385 Pearson correlation analysis (Table 5) and conical correspondence analysis (see
386 Figure 4 in (Shamurad *et al.*, "in press")) between various physiochemical parameters
387 including the concentrations of metal elements in the digestate together with the reactor
388 performances on day 75 were studied. For instance, in reactors that were fed by the LRFM
389 regimen and amended with the MWs (LFA, LBA, LCBW and LIBA) which showed higher
390 and stable biogas production compared to the control, significant correlations (Pearson
391 correlation = 0.945, $p < 0.05$) was found between methane yields and dissolved Mn
392 concentration (Table 5). The data from the DIBA reactor fed using such a different feeding
393 regimen were not included in this correlation analysis and the possible inhibitory impact of
394 Mn in this reactor is discussed in the next section. In addition, an apparent positive
395 correlation (albeit not significant; $p > 0.05$) between the methane yield and single element
396 concentrations like Ni, Mo, Zn, Mg, Co, B and Ba with Pearson correlations of 0.49, 0.33,
397 0.26, 0.315, 0.4, 0.33 and 0.24 were also detected (Table 5). On one hand, significant
398 correlations (Pearson correlations of 0.923 and 0.964, $p < 0.05$) between Co concentrations
399 and both VFA and NH_3-N concentrations were observed. Furthermore, significant
400 correlations (Pearson correlation = 0.967, $p < 0.05$) between NH_3-N concentrations and
401 VFA concentrations, and a positive correlations (Pearson correlation = 0.67 and 0.64, $p >$
402 0.05) between NH_3-N and alkalinity and NH_3-N and pH in the reactors operated on the

LRFM were also detected (Table 5). These results refer to the dual positive effects of the MWs on the microbial activity and alkalinity in the AD reactors. Specifically, some trace elements like Fe, Cu, Zn, Mn, Co, Ni etc. are previously reported to have important roles in the synthesis of coenzymes involved in the metabolic pathways of methanogenesis (Jiang *et al.*; Pobeheim *et al.*, 2010; Demirel and Scherer, 2011; Ünal *et al.*, 2012; Zhang *et al.*, 2015; Westerholm *et al.*, 2016; Cai *et al.*, 2018).

3.4 Assessment of inhibitory and toxicity effects of mineral wastes during anaerobic digestion

Biogas production is an obvious key indicator of the performance and stability of an AD process (Masebinu *et al.*, 2018). Accordingly, the high biogas yields from the SOW in reactors amended with MWs, specifically the reactors fed with LRFM which were expected to contain higher concentrations of metals (Table 2); suggests that there was no obvious inhibitory/toxicity effects from the metals released by the MWs on the microbial activities and hence the biogas production in the reactors (Figure 1) or the relative growth of both bacterial and methanogenic populations in relation to the unamended controls (Figure 2). Dissolved concentration of Cd, Cr, Cu, Ni, Pb, and Zn in the digestates after 75 days of rector operations are shown (see Figure 1 in (Shamurad *et al.*, "in press")). These concentrations were well below the inhibitory thresholds for AD processes (Jiang *et al.*; Hickey *et al.*, 1987; Hickey *et al.*, 1989; Oleszkiewicz and Sharma, 1990; Lin, 1992; Lin, 1993a; Lin, 1993b; Banks and Lo, 2003; Chen *et al.*, 2008; Banks and Zhang, 2010; Banks

423 *et al.*, 2011). The inhibition of AD is expected when the total weight (meq) of the heavy
424 metals Zn, Ni, Pb, Cd and Cu per kg of dry solids in the digesting sludge is ≥ 400 meq/kg
425 (Facchin *et al.*, 2013; Mudhoo and Kumar, 2013; Abdel-Shafy and Mansour, 2014).
426 However, in all the reactors of current study lower magnitudes were detected (1.2, 0.6, 0.6,
427 0.8, 1.7 and 0.85 meq/kg for LFA, LCO, DIBA, LCBW, LBA, and LIBA respectively).
428 Moreover, the results of this study were in line with other research (Lo *et al.*, 2009) which
429 reported that heavy metals released from co-disposal of fly ash with MSW exerted no
430 instability and toxicity effects on the digestion processes.

431 Focusing specifically on manganese, although Cai *et al.* (2018) observed a 48.9%
432 increase in the methane yield from rice straw at a Mn concentration of 1.0 mg/L, they
433 found that acetic acid was accumulated when excessive Mn concentrations were added.
434 This study suggested Mn concentrations were at half-maximal inhibitory concentration
435 (IC50) at 773.9 mg/L. It should be noted here that the dissolved concentration of Mn (5.2
436 mg/L) was found to be considerably higher in the poorer performing DIBA reactor
437 compared to the LRFM reactors (0.66 ± 0.12 mg/L) and the control reactor (0.07 mg/L)
438 which might suggest inhibition by this metal. On balance, a Mn induced reason for the
439 poor performance of the DIBA reactor seems unlikely as levels observed were
440 considerably closer to the stimulatory rather than inhibitory levels determined by (Cai *et*
441 *al.*, 2018).

An alternative indicator of possible negative effects of the mineral wastes is an assessment of their impacts on microbial diversity. This assessment assumes that the toxicity of metals may reduce diversity and select for specific communities tolerant to the imposed conditions. Such effects have been observed in numerous studies of microbial communities (Huang *et al.*, 2003; Nettmann *et al.*, 2008; Nelson *et al.*, 2011; Ünal *et al.*, 2012; Xia *et al.*, 2012; Koch *et al.*, 2013; Wang *et al.*, 2014; Westerholm *et al.*, 2015). In a general sense, the dominant bacterial and archaeal communities in all the reactor communities, regardless of time or treatment were consistent with those that might be expected to proliferate in anaerobic digesters treating food waste for methane production. Evidence for this is provided in the phylogenetic trees shown in Figure 3 and Figure 4 which include close relatives randomly selected from BLAST searches of the Genbank database and in particular their source environments which are dominated by conventional anaerobic digester studies without reports of toxic stress. Furthermore, although the diversity of the microbial communities clearly decreased in the reactors as might be expected from toxicity (see Figure 2 and 3 in (Shamurad *et al.*, "in press")), it was actually operation time and feeding mechanism (i.e. LRFM or DFFM) rather the presence or absence of MWs which were the factors that controlled the dynamics and compositions of the microbial communities. This operational driver for community change was also clear from a principal component analysis of sequence libraries (see Figure 3 and Figure 4 and Figures 2 and 3 in (Shamurad *et al.*, "in press")) where the community compositions (both

462 bacterial and archaeal) in all the reactors was principally influenced by time of operation
463 and not specific amendments. In the inoculum and at 20 days all the communities were
464 dominated by taxa assignable to the candidatus genus *Cloacamonas* (*Cloacimonadaceae*);
465 genus *Thermovirga* (*Synergistaceae*); family *Syntrophomonadaceae*; family
466 *Rikenellaceae;* and (data not shown) family *Bacteroidetes vadinHA17* (see below for a
467 discussion of specific taxa functions). However, by 75 days all the LRFM reactors
468 including the control were dominated by taxa assignable to the *Cloacimonadaceae* W5
469 group; some *Synergistaceae* (not so closely related to the genus *Thermovirga*) and; the
470 genus *Proteiniphilum (Dysgonomonadaceae*). Likewise, in all the LRFM reactors
471 including the control, the archaea underwent substantial changes with a shift from the
472 domination of the genera *Methanosphaera*, Candidatus *Methanoplasma* and
473 *Methanobrevibacter* by 20 days to a general increase in the proportion of archaea and
474 domination of the genera *Methanoculleus* and *Methanosaeta* at day 75. In contrast, by day
475 75 the DIBA reactor (which was also amended with IBA similar to LIBA but was fed with
476 DFFM) was dominated by bacterial taxa assignable to the family *Dysgonomonadaceae*
477 (but unrelated to the genus *Proteiniphilum*) and to a taxon related to the genus *Georgenia*
478 (*Bogoriellaceae*). The archaeal taxa were dominated by the genus *Methanosarcina* with
479 only a minor presence of *Methanosaeta* and only moderate increase in *Methanoculleus.*

3.5 Inferred functions, syntrophic relationships and community selection pressures under different operating conditions

The recent study by Lee *et al.* (2018) has pointed out that taxa such as *Rikenellaceae, Proteiniphilum, Candidatus Cloacimonas, Cloacimonadaceae W5, Bacteroidetes vadinHA17* which were enriched in anaerobic digesters treating food wastewater or sewage sludge are 'known (or suspected) to be' anaerobic mesophilic acetogens. In the case of *Candidatus Cloacimonas* this genus has been implicated in syntrophic partnerships and hydrogen generation from the fermentation of carbohydrates and proteins (Pelletier *et al.*, 2008). Accordingly, the transient (20 days) or ultimate (75 days) enrichment of these groups in the LRFM reactors, coincident with the transient or ultimate enrichment of hydrogenotrophic (*Methanoculleus*) and acetoclastic (*Methanosaeta*) methanogens is entirely consistent with biogas production from the SOW. What is less clear is the reason for the succession between the 20 and 75-day communities with, for instance, the transient dominance of putative methanol reducing and hydrogen oxidising methanogens (*Methanosphaera*, Candidatus *Methanoplasma*) indicating that at 20 days of reactor operation methanol was a major intermediate product of mixed fermentation. Bio-methanol has been observed during the anaerobic co-digestion of animal and agriculture wastes (Anitha *et al.*, 2015). Furthermore, in this study methanol was an early stage product. It has been suggested (Chandra *et al.*, 2012) that products such as

499 methanol are formed in the early phases of continuous or semi-continuous anaerobic
500 digestion because the build-up of acidic products of hydrolysis.

501 By 75 day, the relative dominance of *Methanoculleus* methanogens over
502 *Methanosaeta* suggested the dominance of hydrogenotrophic over acetoclastic
503 methanogenesis indicating the likely occurrence of syntrophic acetate oxidation in the
504 LRFM reactors. *Methanoculleus* spp. have certainly been found in mesophilic syntrophic
505 acetate oxidising digesters (Schnürer *et al.*, 1999; Franke-Whittle *et al.*, 2014; Westerholm
506 *et al.*, 2016), predominating over other hydrogenotrophic methanogens at extreme
507 environmental conditions (i.e. high salt, ammonia and VFA concentrations). In contrast,
508 however, the growth of *Methanosaeta* is known to be sensitive to changes of operational
509 conditions such as VFAs and NH_3-N concentrations (Demirel and Scherer, 2008; Franke-
510 Whittle *et al.*, 2014). That being said a significant positive correlations was found (not
511 shown) between NH_3-N concentrations and both *Methanosaeta* and *Methnoculleous* at day
512 75 and, furthermore, NH_3-N concentrations in the LRFM reactors were below likely
513 inhibitory levels (Westerholm *et al.*, 2015) especially after their substantial decline from
514 the levels measured at 20 days. It can be concluded that the sufficient concentration of
515 NH_3-N in the LRFM reactors supported the growth of microorganisms (Kayhanian, 1999)
516 rather than exerting inhibitory effects.

517 In this study and as discussed above, the control reactor (LCO) had an
518 approximately similar microbial community composition and dynamics to that of the MW

519 amended reactors operated on the same feeding mechanism (LRFM) (Figure 3 and Figure
520 4 and Figures 2 and 3 in (Shamurad *et al.*, "in press")). However, methane production in
521 the LCO reactor decreased gradually and total VFA concentration increased rapidly from
522 340 mg/L on day 46 to about 4073 mg/L on day 73 (Figure 1d) this led to pH drop and a
523 drastic decrease in methane yield. Based on the high degree of similarity in community
524 composition, the low methane production efficiency of the LCO reactor was probably
525 related to two main reasons. Firstly, a lower relative population growth of acetoclastic
526 methanogens (especially *Methanosaeta*; Figure 2d and Figure 4) in this reactor compared
527 to the MW amended reactors (specifically LCBW, LBA and LIBA). Secondly, low trace
528 element concentrations in the LCO reactor affected methanogenic activity in this reactor
529 especially when the OLR was increased to 1 g VS/L.d. The deficiency of the required trace
530 elements in the SOW substrate caused an alteration in methanogenic pathways and a
531 decline in digestion performance (Westerholm *et al.*, 2015), since this decrease in
532 methanogenesis was not observed in the other reactors that were operated on LRFM and
533 amended with the MWs (i.e. LFA, LCBW, LBA, and LIBA). In contrast, in the DIBA
534 reactor a low NH_3-N concentration was existed, therefore presumably the growth of
535 *Methanosarcina* (which is known for its high growth rates and dominance when high
536 levels of VFA present (Franke-Whittle *et al.*, 2014)) was limited due to the lack of enough
537 N nutrient needed for the population growth.

The dominance by day 75 of very different bacterial and archaeal taxa in the DIBA reactor fed by DFFM feeding regimen was likely dictated by the prevailing conditions within this reactor. With respect to the bacterial sequences enriched, a taxon closely related to the genus *Georgenia* (family *Bogoriellaceae*) is notable as isolates of this genus range from aerobic, microaerophilic to facultative anaerobic metabolisms (Ward and Bora, 2009) and this genus does not appear to be a commonly associated with anaerobic digestion. However, a close relative has identified as a dominant component of the granular sludge of a low temperature glucose fed anaerobic digester (O'Reilly *et al.*, 2010) in a reactor where the dominant methanogen was the putative hyrdrogenotroph *Methanocorpusculum*. However, in contrast, in the present study this substantial enrichment of the *Georgenia* taxon along with a taxon from the family *Dysgonomonadaceae* in the DIBA reactor was associated with enrichment of the methanogenic genus *Methanosarcina* which is also known to be metabolically more versatile and robust with shorter doubling times and tolerance to environmental stress such as low pH (Calli *et al.*, 2005; Conklin *et al.*, 2006; Thauer *et al.*, 2008). Several previous studies have linked *Methanosarcinaceae*-related populations to high residual acetate concentrations often associated with poor COD removal (Hulshoff Pol *et al.*, 2004). However, high COD, VFA and low pH was actually a property of the LCO reactor which sustained a similar microbial community to all other reactors which included the presence of *Methanosaeta* typically considered less tolerant to such stresses. The most obvious distinguishing feature of the DIBA reactor in comparison

to all the other reactors was the relatively low NH_3-N concentrations which is of interest because it is another selection factor for *Methanosarcina* , since previous studies have reported the predominance of *Methanosarcina* at high ammonia concentrations (Calli *et al.*, 2005; Tian *et al.*, 2018).

4. Conclusion

1- The MWs from MSWI plants and CDW can be utilised as trace element supplements for optimising (high biogas production and stable digestion process) the AD of organic materials.
2- The metals released from the MWs enhanced the buffering capacity and metabolic/catabolic activities in the AD reactors without inhibitory/toxicity effects.
3- The LRFM feeding method can be considered as a proper feeding method for anaerobic co-digestion of OFMSW with MWs from MSWI plants and CDW.
4- Feeding methods and time were the key factors affecting microbial diversity in AD reactors supplemented with or without the MWs.

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578 Declaration on conflict of interest

579 We declare that there is no conflict of interest.

References

Abdel-Shafy, H.I. and Mansour, M.S.M. (2014) 'Biogas production as affected by heavy metals in the anaerobic digestion of sludge', *Egyptian Journal of Petroleum*, 23(4), pp. 409-417.

Akay, G., Erhan, E. and Keskinler, B. (2005) 'Bioprocess intensification in flow-through monolithic microbioreactors with immobilized bacteria', *Biotechnology and Bioengineering*, 90(2), pp. 180-190.

Anitha, M., Kamarudin, S.K., Shamsul, N.S. and Kofli, N.T. (2015) 'Determination of bio-methanol as intermediate product of anaerobic co-digestion in animal and agriculture wastes', *International Journal of Hydrogen Energy*, 40(35), pp. 11791-11799.

AWWA (2012) *Standard methods for examination of water and wastewater*. 22nd ed.. edn. Washington, D.C.: Washington, D.C. : American Water Works Association.

Banks, C.J. and Lo, H.-M. (2003) 'Assessing the effects of municipal solid waste incinerator bottom ash on the decomposition of biodegradable waste using a completely mixed anaerobic reactor', *Waste Management and Research*, 21(3), pp. 225-234.

Banks, C.J. and Zhang, Y. (2010) 'Technical Report: Optimising inputs and outputs from anaerobic digestion processes', *Defra Project Code WR0212*.

Banks, C.J., Zhang, Y., Jiang, Y. and Heaven, S. (2011) 'Trace element requirements for stable food waste digestion at elevated ammonia concentrations', *Bioresource Technology*, 104, pp. 127-135.

Berlanga Herranz, M. (2008) 'Brock Biology of microorganisms 12th edn', *International microbiology: official journal of the Spanish Society for Microbiology*, (1), pp. 65-66.

Cai, Y., Zheng, Z., Zhao, Y., Zhang, Y., Guo, S., Cui, Z. and Wang, X. (2018) 'Effects of molybdenum, selenium and manganese supplementation on the performance of anaerobic digestion and the characteristics of bacterial community in acidogenic stage', *Bioresource technology*, 266, pp. 166-175.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. and Holmes, S.P. (2016) 'DADA2: high-resolution sample inference from Illumina amplicon data', *Nature methods*, 13(7), p. 581.

Calli, B., Mertoglu, B., Inanc, B. and Yenigun, O. (2005) 'Methanogenic diversity in anaerobic bioreactors under extremely high ammonia levels', *Enzyme and Microbial Technology*, 37(4), pp. 448-455.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K. and Gordon, J.I. (2010) 'QIIME allows analysis of high-throughput community sequencing data', *Nature methods*, 7(5), p. 335.

Chandler, A.J., Eighmy, T.T., Hjelmar, O., Kosson, D.S., Sawell, S.E., Vehlow, J., Van der Sloot, H.A. and Hartlén, J. (1997) *Municipal solid waste incinerator residues*. Elsevier.

617 Chandra, R., Vijay, V.K., Subbarao, P.M.V. and Khura, T.K. (2012) 'Production of
618 methane from anaerobic digestion of jatropha and pongamia oil cakes', *Applied Energy*, 93,
619 pp. 148-159.
620 Chen, Y., Cheng, J.J. and Creamer, K.S. (2008) 'Inhibition of anaerobic digestion process:
621 A review', *Bioresource Technology*, 99(10), pp. 4044-4064.
622 Chimenos, J., Segarra, M., Fernandez, M. and Espiell, F. (1999) 'Characterization of the
623 bottom ash in municipal solid waste incinerator', *Journal Of Hazardous Materials*, 64(3),
624 pp. 211-222.
625 Conklin, A., Stensel, H.D. and Ferguson, J. (2006) 'Growth kinetics and competition
626 between Methanosarcina and Methanosaeta in mesophilic anaerobic digestion', *Water*
627 *Environment Research*, 78(5), pp. 486-496.
628 Dai, X., Duan, N., Dong, B. and Dai, L. (2013) 'High-solids anaerobic co-digestion of
629 sewage sludge and food waste in comparison with mono digestions: stability and
630 performance', *Waste management (New York, N.Y.)*, 33(2), p. 308.
631 DEFRA (2018) *UK Statistics on Waste*. [Online]. Available at:
632 https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_d
633 ata/file/683051/UK_Statisticson_Waste_statistical_notice_Feb_2018_FINAL.pdf
634 (Accessed: April 2017).
635 Demirel, B. and Scherer, P. (2008) 'The roles of acetotrophic and hydrogenotrophic
636 methanogens during anaerobic conversion of biomass to methane: a review', *Reviews in*
637 *Environmental Science and Bio/Technology*, 7(2), pp. 173-190.
638 Demirel, B. and Scherer, P. (2011) 'Trace element requirements of agricultural biogas
639 digesters during biological conversion of renewable biomass to methane', *Biomass and*
640 *Bioenergy*, 35(3), pp. 992-998.
641 Dong, B., Liu, X., Dai, L. and Dai, X. (2013) 'Changes of heavy metal speciation during
642 high-solid anaerobic digestion of sewage sludge', *Bioresource Technology*, 131, pp. 152-
643 158.
644 El-Mashad, H.M. and Zhang, R. (2010) 'Biogas production from co-digestion of dairy
645 manure and food waste', *Bioresource technology*, 101(11), pp. 4021-4028.
646 eurostat (2018) 'Municipal waste statistics'. Available at:
647 https://ec.europa.eu/eurostat/statistics-
648 explained/index.php?title=Municipal_waste_statistics.
649 Facchin, V., Cavinato, C., Fatone, F., Pavan, P., Cecchi, F. and Bolzonella, D. (2013)
650 'Effect of trace element supplementation on the mesophilic anaerobic digestion of
651 foodwaste in batch trials: The influence of inoculum origin', *Biochemical Engineering*
652 *Journal*, 70, pp. 71-77.
653 Feng, X., Karlsson, A., Svensson, B. and Bertilsson, S. (2010) 'Impact of trace element
654 addition on biogas production from food industrial waste - linking process to microbial
655 communities', *Fems Microbiology Ecology*, 74(1), pp. 226-240.

Forster-Carneiro, T., Pérez, M. and Romero, L.I. (2008) 'Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste', *Bioresource Technology*, 99(15), pp. 6994-7002.
Franke-Whittle, I.H., Walter, A., Ebner, C. and Insam, H. (2014) 'Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities', *Waste Management*, 34(11), pp. 2080-2089.
Griffiths, R., Whiteley, A., O'Donnell, A.G. and Bailey, M. (2000) 'Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition', *Applied And Environmental Microbiology*, 66(12), pp. 5488-5491.
Gu, X. and Wong, J.W.C. (2004) 'Identification of Inhibitory Substances Affecting Bioleaching of Heavy Metals from Anaerobically Digested Sewage Sludge', *Environmental Science & Technology*, 38(10), pp. 2934-2939.
Hamilton, J.J., Contreras, M.C. and Reed, J.L. (2015) 'Thermodynamics and H2 transfer in a methanogenic, syntrophic community', *PLoS computational biology*, 11(7), p. e1004364.
Harms, G., Layton, A.C., Dionisi, H.M., Gregory, I.R., Garrett, V.M., Sayler, G.S., Hawkins, S.A. and Robinson, K.G. (2003) 'Real-time PCR quantification of nitrifying bacteria in a municipal wastewater treatment plant', *Environmental Science and Technology*, 37(2), pp. 343-351.
Hickey, R., Vanderwielen, J. and Switzenbaum, R. (1989) 'The effect of heavy metals on methane production and hydrogen and carbon monoxide levels during batch anaerobic sludge digestion', *Water Research*, 23(2), pp. 207-218.
Hickey, R.F., Vanderwielen, J. and Switzenbaum, M.S. (1987) 'The effects of organic toxicants on methane production and hydrogen gas levels during the anaerobic digestion of waste activated sludge', *Water Research*, 21(11), pp. 1417-1427.
Huang, L.-N., Chen, Y.-Q., Zhou, H., Luo, S., Lan, C.-Y. and Qu, L.-H. (2003) 'Characterization of methanogenic Archaea in the leachate of a closed municipal solid waste landfill', *FEMS Microbiology Ecology*, 46(2), pp. 171-177.
Hude Moreshwar, P. and Yadav Ganapati, D. (2014) 'Process intensification in methane generation during anaerobic digestion of Napier grass using supercritical carbon dioxide combined with acid hydrolysis pre-treatment', *The Canadian Journal of Chemical Engineering*, 92(12), pp. 2176-2184.
Hulshoff Pol, L.W., de Castro Lopes, S.I., Lettinga, G. and Lens, P.N.L. (2004) 'Anaerobic sludge granulation', *Water Research*, 38(6), pp. 1376-1389.
IBM, C. (2015) *IBM SPSS Statistics for Windows, Version 23.0* [Computer program]. Armonk, NY: IBM Corp.
Jiang, Y., Zhang, Y., Banks, C., Heaven, S. and Longhurst, P. 'Investigation of the impact of trace elements on anaerobic volatile fatty acid degradation using a fractional factorial experimental design', *Water Research*.

695 Kayhanian, M. (1999) 'Ammonia inhibition in high-solids biogasification: an overview and
696 practical solutions', *Environmental Technology*, 20(4), pp. 355-365.
697 Klappenbach, J.A., Saxman, P.R., Cole, J.R. and Schmidt, T.M. (2001) 'rrndb: the
698 ribosomal RNA operon copy number database', *Nucleic acids research*, 29(1), pp. 181-
699 184.
700 Koch, C., Günther, S., Desta, A.F., Hübschmann, T. and Müller, S. (2013) 'Cytometric
701 fingerprinting for analyzing microbial intracommunity structure variation and identifying
702 subcommunity function', *Nature Protocols*, 8(1), pp. 190-202.
703 Koster, I.W. and Lettinga, G. (1984) 'The influence of ammonium-nitrogen on the specific
704 activity of pelletized methanogenic sludge', *Agricultural Wastes*, 9(3), pp. 205-216.
705 Kumar, S., Stecher, G. and Tamura, K. (2016) 'MEGA7: molecular evolutionary genetics
706 analysis version 7.0 for bigger datasets', *Molecular biology and evolution*, 33(7), pp. 1870-
707 1874.
708 Lee, J., Kim, E., Han, G., Tongco, J.V., Shin, S.G. and Hwang, S. (2018) 'Microbial
709 communities underpinning mesophilic anaerobic digesters treating food wastewater or
710 sewage sludge: A full-scale study', *Bioresource Technology*, 259, pp. 388-397.
711 Liebetrau, J., Sträuber, H., Kretzschmar, J., Denysenko, V. and Nelles, M. (2017)
712 'Anaerobic Digestion', *Advances in biochemical engineering/biotechnology*.
713 Lin, C.-Y. (1992) 'Effect of heavy metals on volatile fatty acid degradation in anaerobic
714 digestion', *Water Research*, 26(2), pp. 177-183.
715 Lin, C.-Y. (1993a) 'Effect of heavy metals on acidogenesis in anaerobic digestion', *Water*
716 *Research*, 27(1), pp. 147-152.
717 Lin, C. (1993b) 'EFFECT OF HEAVY-METALS ON ACIDOGENESIS IN
718 ANAEROBIC-DIGESTION', *Water Research*, 27(1), pp. 147-152.
719 Liu, A., Ren, F., Lin, W.Y. and Wang, J.-Y. (2015) 'A review of municipal solid waste
720 environmental standards with a focus on incinerator residues', *International Journal of*
721 *Sustainable Built Environment*, 4(2), pp. 165-188.
722 Lo, H.-M. (2005) 'Metals behaviors of MSWI bottom ash co-digested Anaerobically with
723 MSW', *Resources, Conservation and Recycling*, 43(3), pp. 263-280.
724 Lo, H.M., Liu, M.H., Pai, T.Y., Liu, W.F., Lin, C.Y., Wang, S.C., Banks, C.J., Hung, C.H.,
725 Chiang, C.F., Lin, K.C., Chen, P.H., Chen, J.K., Chiu, H.Y., Su, M.H., Kurniawan, T.A.,
726 Wu, K.C., Hsieh, C.Y. and Hsu, H.S. (2009) 'Biostabilization assessment of MSW co-
727 disposed with MSWI fly ash in anaerobic bioreactors', *Journal of Hazardous Materials*,
728 162(2), pp. 1233-1242.
729 Lossie, U. and Pütz, P. (2008) 'Targeted control of biogas plants with the help of
730 FOS/TAC', *Practice Report Hach-Lange*.
731 Masebinu, S.O., Akinlabi, E.T., Muzenda, E., Aboyade, A.O. and Mbohwa, C. (2018)
732 'Experimental and feasibility assessment of biogas production by anaerobic digestion of
733 fruit and vegetable waste from Joburg Market', *Waste Management*, 75, pp. 236-250.

McMurdie, P. and Holmes, S. (2013) 'phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data', *PLoS One*, 8(4), p. e61217.
Mudhoo, A. and Kumar, S. (2013) 'Effects of heavy metals as stress factors on anaerobic digestion processes and biogas production from biomass', *International Journal of Environmental Science and Technology : (IJEST)*, 10(6), pp. 1383-1398.
Nelson, M.C., Morrison, M. and Yu, Z. (2011) 'A meta-analysis of the microbial diversity observed in anaerobic digesters', *Bioresource Technology*, 102(4), pp. 3730-3739.
Nettmann, E., Bergmann, I., Mundt, K., Linke, B. and Klocke, M. (2008) 'Archaea diversity within a commercial biogas plant utilizing herbal biomass determined by 16S rDNA and mcrA analysis', *Journal of Applied Microbiology*, 105(6), pp. 1835-1850.
Nielfa, A., Cano, R. and Fdz-Polanco, M. (2015) 'Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge', *Biotechnology Reports*, 5, pp. 14-21.
O'Reilly, J., Lee, C., Chinalia, F., Collins, G., Mahony, T. and O'Flaherty, V. (2010) 'Microbial community dynamics associated with biomass granulation in low-temperature (15°C) anaerobic wastewater treatment bioreactors', *Bioresource Technology*, 101(16), pp. 6336-6344.
Oleszkiewicz, J.A. and Sharma, V.K. (1990) 'Stimulation and inhibition of anaerobic processes by heavy metals—A review', *Biological Wastes*, 31(1), pp. 45-67.
Parks, D.H., Tyson, G.W., Hugenholtz, P. and Beiko, R.G. (2014) 'STAMP: statistical analysis of taxonomic and functional profiles', *Bioinformatics*, 30(21), pp. 3123-3124.
Pelletier, E., Kreimeyer, A., Bocs, S., Rouy, Z., Gyapay, G., Chouari, R., Rivière, D., Ganesan, A., Daegelen, P. and Sghir, A. (2008) '"Candidatus Cloacamonas acidaminovorans": genome sequence reconstruction provides a first glimpse of a new bacterial division', *Journal of bacteriology*, 190(7), pp. 2572-2579.
Petropoulos, E., Dolfing, J., Davenport, R.J., Bowen, E.J. and Curtis, T.P. (2017) 'Developing cold-adapted biomass for the anaerobic treatment of domestic wastewater at low temperatures (4, 8 and 15 °C) with inocula from cold environments', *Water Research*, 112(C), pp. 100-109.
Pobeheim, H., Munk, B., Johansson, J. and Guebitz, G. (2010) 'Influence of trace elements on methane formation from a synthetic model substrate for maize silage', *Bioresource Technology*, 101(2), pp. 836-839.
Renforth, P., Washbourne, C., Taylder, J. and Manning, D. (2011) 'Silicate Production and Availability for Mineral Carbonation', *Environmental Science & Technology*, 45(6), pp. 2035-2041.
Rognes, T., Flouri, T., Nichols, B., Quince, C. and Mahé, F. (2016) 'VSEARCH: a versatile open source tool for metagenomics', *PeerJ*, 4, p. e2584.
Saitou, N. and Nei, M. (1987) 'The neighbor-joining method: a new method for reconstructing phylogenetic trees', *Molecular biology and evolution*, 4(4), pp. 406-425.

SCA (1986) *Methods for the Determination of Metals in Soils, Sediments and Sewage Sludge and Plants by Hydrochloric-Nitric Acid Digestion*. London: Standing Committee of Analysts.
Schnürer, A., Zellner, G. and Svensson, B.H. (1999) 'Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors', *FEMS Microbiology Ecology*, 29(3), pp. 249-261.
Shamurad, B., Gray, N., Petropoulos, E., Tabraiz, S., Acharya, K., Quintela-Baluja, M. and Sallis, P. ("in press") 'Metal and microbial analyses of co-digestion of organic and mineral wastes in mesophilic anaerobic digestion', *Data in Brief*, "in press".
Steinberg, L.M. and Regan, J.M. (2009) 'mcrA-Targeted Real-Time Quantitative PCR Method To Examine Methanogen Communities', *Applied and Environmental Microbiology*, 75(13), p. 4435.
Takashima, M., Shimada, K. and Speece, R. (2011) 'Minimum Requirements for Trace Metals (Iron, Nickel, Cobalt, and Zinc) in Thermophilic and Mesophilic Methane Fermentation from Glucose', *Water Environment Research*, 83(4), pp. 339-46.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) 'MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods', *Molecular biology and evolution*, 28(10), pp. 2731-2739.
Thauer, R.K., Kaster, A.-K., Seedorf, H., Buckel, W. and Hedderich, R. (2008) 'Methanogenic archaea: ecologically relevant differences in energy conservation', *Nature Reviews Microbiology*, 6(8), p. 579.
Tian, H., Fotidis, I.A., Kissas, K. and Angelidaki, I. (2018) 'Effect of different ammonia sources on aceticlastic and hydrogenotrophic methanogens', *Bioresource Technology*, 250, pp. 390-397.
Ünal, B., Perry, V.R., Sheth, M., Gomez-Alvarez, V., Chin, K.J. and Nüsslein, K. (2012) 'Trace elements affect methanogenic activity and diversity in enrichments from subsurface coal bed produced water', *Front Microbiol*, 3.
VDI (2006) 'VDI 4630 – Fermentation of Organic Materials Characterisation of the Substrate, Sampling, Collection of Material Data, Fermentation Tests'.
Viswanath, P., Devi, S.S. and Nand, K. (1992) 'Anaerobic digestion of fruit and vegetable processing wastes for biogas production', *Bioresource technology*, 40(1), pp. 43-48.
Wang, C., Zuo, J., Chen, X., Xing, W., Xing, L., Li, P., Lu, X. and Li, C. (2014) 'Microbial community structures in an integrated two-phase anaerobic bioreactor fed by fruit vegetable wastes and wheat straw', *Journal of Environmental Sciences*, 26(12), pp. 2484-2492.
Ward, A.C. and Bora, N.M. (2009) *27 The Actinobacteria*. Second edn.
Ward, A.J., Hobbs, P.J., Holliman, P.J. and Jones, D.L. (2008) 'Optimisation of the anaerobic digestion of agricultural resources', *Bioresource technology*, 99(17), pp. 7928-7940.

Washbourne, C., Lopez-Capel, E., Renforth, P., Ascough, P. and Manning, D. (2015) 'Rapid Removal of Atmospheric CO2 by Urban Soils', *Environmental Science & Technology*, 49(9), pp. 5434-5440.
Westerholm, M., Moestedt, J. and Schnürer, A. (2016) 'Biogas production through syntrophic acetate oxidation and deliberate operating strategies for improved digester performance', *Applied Energy*, 179, pp. 124-135.
Westerholm, M., Muller, B., Isaksson, S. and Schnurer, A. (2015) 'Trace element and temperature effects on microbial communities and links to biogas digester performance at high ammonia levels', *Biotechnology for Biofuels*, 8.
Westerlund, J. and Edgerton, D.L. (2007) 'A panel bootstrap cointegration test', *Economics Letters*, 97(3), pp. 185-190.
Wu, L.-J., Kobayashi, T., Kuramochi, H., Li, Y.-Y. and Xu, K.-Q. (2016) 'Effects of potassium, magnesium, zinc, and manganese addition on the anaerobic digestion of de-oiled grease trap waste', *Arabian Journal for Science and Engineering*, 41(7), pp. 2417-2427.
Xia, Y., Massé, D.I., McAllister, T.A., Kong, Y., Seviour, R. and Beaulieu, C. (2012) 'Identity and diversity of archaeal communities during anaerobic co-digestion of chicken feathers and other animal wastes', *Bioresource Technology*, 110, pp. 111-119.
Zandvoort, M., van Hullebusch, E., Fermoso, F. and Lens, P. (2006) 'Trace metals in anaerobic granular sludge reactors: Bioavailability and dosing strategies', *Engineering In Life Sciences*, 6(3), pp. 293-301.
Zhang, L., Lee, Y. and Jahng, D. (2011) 'Anaerobic co-digestion of food waste and piggery wastewater: Focusing on the role of trace elements', *Bioresource Technology*, 102(8), pp. 5048-5059.
Zhang, L., Ouyang, W. and Lia, A. (2012) 'Essential Role of Trace Elements in Continuous Anaerobic Digestion of Food Waste', *Procedia Environmental Sciences*, 16, pp. 102-111.
Zhang, W., Zhang, L. and Li, A. (2015) 'Enhanced anaerobic digestion of food waste by trace metal elements supplementation and reduced metals dosage by green chelating agent [S, S]-EDDS via improving metals bioavailability', *Water research*, 84, pp. 266-277.
Zhu, H., Parker, W., Basnar, R., Proracki, A., Falletta, P., Béland, M. and Seto, P. (2008) 'Biohydrogen production by anaerobic co-digestion of municipal food waste and sewage sludges', *International Journal of Hydrogen Energy*, 33(14), pp. 3651-3659.

Figure legends

Figure 1. Profiles of methane yield (A), methane accumulation (B), total alkalinity (C) and total volatile fatty acids (D) during the single-stage co-digestion of synthetic organic waste and mineral wastes from MSWI plants and cement-based waste (IBA=incineration bottom ash, FA=fly ash, BA=boiler ash, CBW=cement-based waste) in comparison to mineral free control. L and D indicate the reactors feeding method liquid-recycled feeding method and draw-and-fill feeding method respectively. The values for total alkalinity and total VFA are mean values of triplicate samples with standard deviations (not shown).

Figure 2. Microbial gene abundances for bacteria ((A) and (B)) and methanogens ((C) and (D)) in the inoculum on day 0 and digestates on day 20 and 75 calculated from qPCR analyses. Error bars represent standard deviations of microbial gene abundances calculated for triplicate samples from qPCR analyses.

Figure 3. Phylogenetic distance tree (Neighbour-Joining) of key AD reactor bacterial taxa and close relatives (left) and plots of the fractional abundances of these taxa in individual reactor sequence libraries (right). The tree is based on comparative analysis of selected partial 16S rRNA sequences recovered from the anaerobic reactors at day 20 and 75 and indicated by individual codes assigned during pipeline analysis. The percentage of replicate trees in which the associated taxa clustered together in bootstrap analysis (1000 replicates) are shown next to the branches. The analysis involved 252 nucleotide positions.

Figure 4. Phylogenetic distance tree (Neighbour-Joining) of key AD reactor archaeal taxa and close relatives (left) and, plots of the fractional abundances of these taxa in individual reactor sequence libraries (right). The tree is based on comparative analysis of selected partial 16S rRNA sequences recovered from the anaerobic reactors at day 20 and 75 and indicated by individual codes assigned during pipeline analysis. The percentage of replicate trees in which the associated taxa clustered together in bootstrap analysis (1000 replicates) are shown next to the branches. The analysis involved 252 nucleotide positions.

881 # Nomenclature

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Symbol	Description	Symbol	Description
AD	Anaerobic digestion/digester	**MSW**	Municipal solid waste
ANOVA	Analysis of Variance	**MSWI**	Municipal solid waste incineration
BA	Boiler ash	**MW**	Mineral wastes
BFA	Bag filter ash	**NH_3-N**	Ammonia nitrogen
BMP	Bio-methane potential	**OFMSW**	Organic fraction of municipal solid waste
C	Celsius	**OLR**	Organic loading rate
CBW	Cement- based waste	**p**	p value (calculated probability)
CDW	Construction demolition wastes	**pgram**	pico gram
CH_4	Methane	**pmol**	pico mol
CO	Control reactor	**qPCR**	Quantitative polymerase chain reaction
CO_2	Carbon dioxide	**rpm**	Revolutions per minute
COD	Chemical oxygen demand	**rRNA**	Ribosomal ribonucleic acid
CSTR	Continually stirred anaerobic reactor	**sCOD**	Soluble chemical oxygen demand
DFFM	Draw-and-fill feeding method	**SD**	Standard deviation
DNA	Deoxyribonucleic acid	**Sig.**	Significant
DW	Distilled water	**SOW**	Synthetic organic waste
GC	Gas chromatograph	**TALK**	Total alkalinity
HCL	Hydrochloric acid	**tCOD**	Total chemical oxygen demand
HNO_3	Nitric acid	**TKN**	Total Kjeldahl Nitrogen
HRT	Hydraulic retention time	**TS**	Total solids
IBA	Incineration bottom ash	**TVFA**	Total volatile fatty acids
LRFM	Liquid recycled feeding method	**VS**	Volatile solids

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Table 1. Characteristics of the inoculum used to start up the CSTR systems*

Parameters	**Characteristics of inoculum**
pH	8.2 ± 0.3
TS (%w/w)**	1.3 ± 0.1
VS (%w/w**)**	0.7 ± 0.1
VS (%TS**)**	52.0
Total TKN (mg. L^{-1}**)**	2848 ± 171.0
NH_3-N (mg. L^{-1}**)**	2654 ± 6.0
FAN (mg. L^{-1}**)**	449 ± 1.0
Total alkalinity (mg. L^{-1}**)**	9792 ± 157.0
Total VFA (mg. L^{-1}**)**	3700 ± 518.0
Total COD (mg. L^{-1}**)**	8100 ± 225.0

*All values in this table represent mean value of triplicate samples measured ± standard deviation of values. ** (%w/w) = percentage of the dry weight of solids per wet weight of digestate.

910 **Table 2**. Physicochemical characteristics of the organic waste and mineral wastes*

Parameters	Synthetic organic waste	Incineration bottom ash	Fly ash	Boiler ash	Cement based waste
pH (1:2**)**	4.3 ± 0.1	10.4 ± 0.5	10.0 ± 0.4	12.0 ± 0.2	11.0 ± 0.7
TS (%W/W**)**	18.6 ± 0.1	99 ± 0.1	97 ± 0.1	100 ± 0.1	97. ± 0.1
VS (%W/W**)**	17 ± 0.1	2.86 ± 0.03	3 ± 0.04	1.2 ± 0.2	2.44 ± 0.03
VS (%TS**)**	92	2.9	3	1	3
C (%**)**	46.47 ± 0.3	1.77 ± 0.5	3 ± 0.13	1.4 ± 0.1	2.7 ± 0.2
H (%**)**	6.76 ± 0.04	-	-	-	-
N (%**)**	2.21 ± 0.02	0.04 ± 0.02	0.02 ± 0.001	0.01 ± 0.002	0.02 ± 0.01
O (%**)**	37.52 ± 0.4	-	-	-	-
S (%**)**	0.16 ± 0.01	0.41 ± 0.1	2 ± 0.023	2.3 ± 0.11	0.13 ± 0.02
C/N	21.0 ± 0.05	50 ± 0.3	2.3 ± 0.12	104 ± 12	139 ± 16.0
Total alkalinity [1]	-	2.6 ± 0.1	5.3 ± 0.18	6 ± 0.3	6.7 ± 0.3
Ignition loss (%**)**	-	3.0 ± 0.1	3.0 ± 0.01	1.0 ± 0.005	3.0 ± 0.1
Al**	45 ± 23.0	30567 ± 842.0	15717 ± 85.0	37587.5 ± 260	11201 ± 251
As	0.61 ± 0.3	7.2 ± 3.8	56 ± 4.5	47 ± 4.8	3.9 ± 0.6
B	4.2 ± 1.2	69 ± 43.0	57 ± 14.0	109 ± 12.0	5.2 ± 1.0
Ba	3.50 ± 0.9	159 ± 10.0	144 ± 9.0	71 ± 6.0	127 ± 10.0
Ca	4958 ± 245	81155 ± 432.0	229861 ± 667	200137 ± 1029	138461 ± 699
Cd	0.02 ± 0.001	5.6 ± 2.0	161 ± 22.0	67 ± 10.0	0.2 ± 0.1
Co	0.03 ± 0.001	49 ± 7.3	12 ± 1.3	21 ± 1.0	5.5 ± 1.5
Cr	0.9 ± 0.2	104 ± 1.9	42 ± 4.0	103 ± 4.0	33 ± 0.2
Cu	4.4 ± 0.7	2772 ± 99.0	591 ± 23.0	529 ± 17.0	17 ± 0.3
Fe	63 ± 4.0	91226 ± 660.0	7011 ± 52.0	13267 ± 116	19883 ± 176
K	7523 ± 1220	3234 ± 39.0	38923 ± 1829	21647 ± 1251	1559 ± 3.4
Mg	657 ± 143.0	7330 ± 355.0	6216 ± 190	13777 ± 411	11388 ± 491
Mn	12 ± 2.0	1302 ± 59.0	384 ± 4.0	1276 ± 13.0	357 ± 8.0
Mo	0.48 ± 0.21	7 ± 1.0	14 ± 6.6	20 ± 7.0	1.2 ± 5.4
Na	323 ± 82.0	246 ± 94.0	198.5 ± 10.0	347 ± 132.0	37.3 ± 12.0
Ni	0.73 ± 0.2	123 ± 1.4	86 ± 0.39	136 ± 1.3	12.8 ± 0.5
Pb	0.28 ± 0.05	1067 ± 10.0	2075 ± 10.0	974 ± 10.0	15.5 ± 0.1
Se	0.85 ± 0.2	13.7 ± 0.1	2.8 ± 18.8	3.4 ± 1.0	2.9 ± 0.1
Si	69 ± 3.0	72 ± 7.0	234.4 ± 2.5	82 ± 1.7	72.4 ± 2.0
Ti	0.40 ± 0.1	1822 ± 15.0	1782 ± 15	230 ± 0.8	791 ± 8.0
V	1.1 ± 0.07	191 ± 19.0	478 ± 39.0	621 ± 48.0	31 ± 29.0
Zn	14 ± 2.5	3421 ± 9.0	11355 ± 44.0	8885 ± 52.0	56 ± 8.0

911 * All values in this table represent mean value of triplicate samples measured ± standard deviation of the values. ** All
912 concentrations are total concentration of metals in mg per kg TS of MWs. [1] Unit = **(**meq. $g^{-1}CaCO_3$**)**

913 **Table 3.** Experimental design of the CSTR systems

Reactor ID	Feeding method	Mineral waste added
LFA	LRFM*	Fly ash (FA)
LCO	LRFM	Control reactor (no MW added)
DIBA	DFFM**	Incineration bottom ash (IBA)
LCBW	LRFM	Cement-based waste (CBW)
LBA	LRFM	Boiler ash (BA)
LIBA	LRFM	Incineration bottom ash (IBA)

914 *LRFM = liquid-recycled feeding method, **DFFM = draw-and- fill feeding method.

Table 4. Summary of reactor parameters on day 20 and day 75*

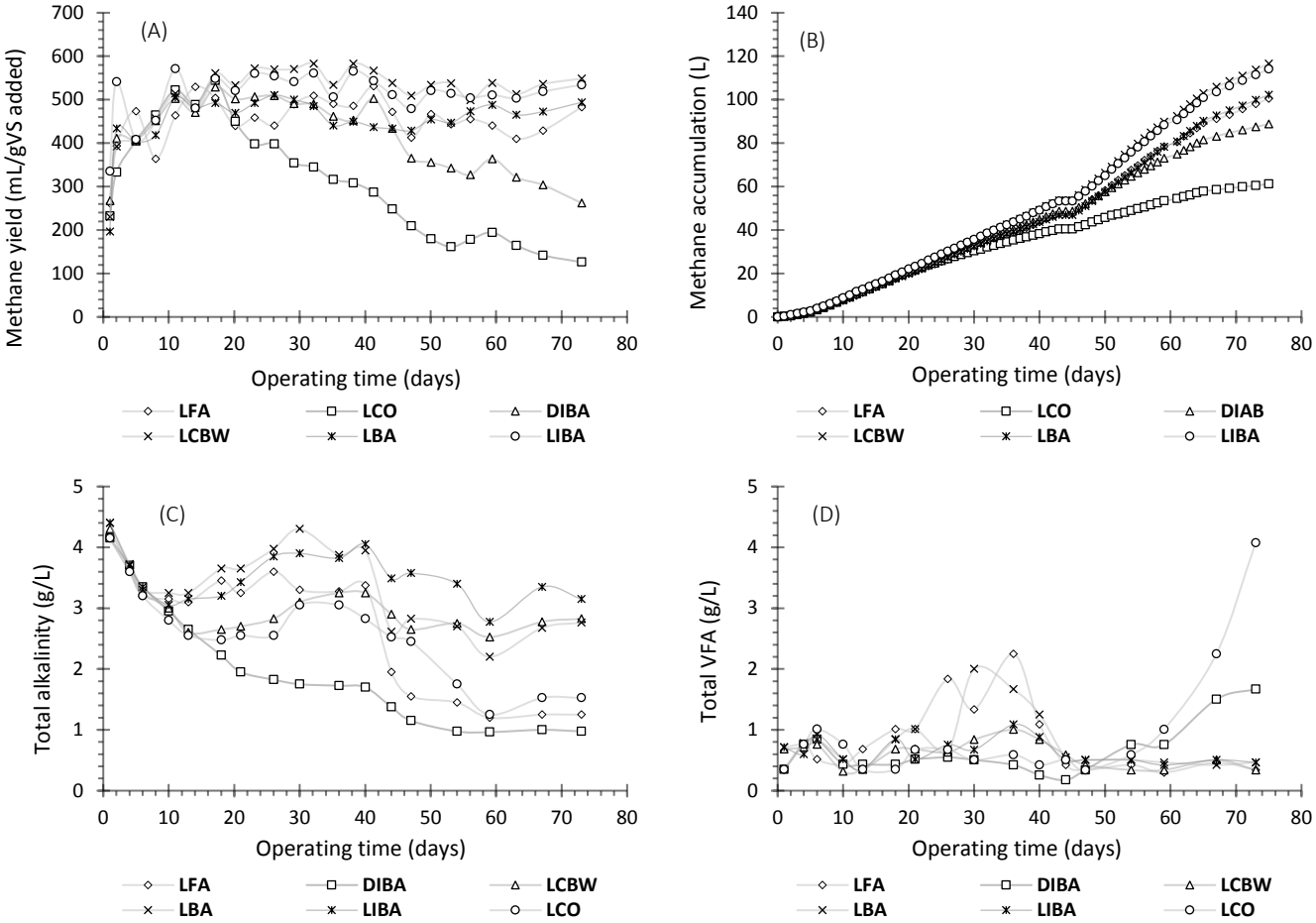
	Reactors	Methane Yield [1]	Hydrolysis activity [2] **	Methanogenesis activity [3]	Mixed liquor soluble COD [4] ***	pH	Mixed liquor NH_3-N [5]
Day 20	**LCO**	484	-	-	2831 ± 516	6.99 ± 0.1	538 ± 59
	LFA	476	-	-	4502 ± 1284	6.7 ± 0.1	521 ± 23
	DIBA	476	-	-	4014 ± 687	6.7 ± 0.1	447 ± 50
	LCBW	558	-	-	4194 ± 788	6.9 ± 0.1	539 ± 36
	LBA	464	-	-	4647 ± 534	6.9 ± 0.1	559 ± 36
	LIBA	536		-	5272 ± 758	7.1 ± 0.05	595 ± 38
Day 75	**LCO**	219	0.068	0.015	3619 ± 1020	5.8 ± 0.3	378 ± 14
	LFA	454	0.081	0.049	4250 ± 742	6.4 ± 0.03	410 ± 8
	DIBA	286	0.106	0.363	3630 ± 1270	5.7 ± 0.2	33 ± 27
	LCBW	536	0.033	0.016	2625 ± 625	6.9 ± 0.03	399 ± 11
	LBA	480	0.087	0.017	3940 ± 860	6.8 ± 0.02	402 ± 14
	LIBA	522	0.028	0.014	2850 ± 450	7.0 ± 0.01	477 ± 1

*All values are mean values for triplicate samples with standard deviation. ** Methanogenesis and hydrolysis activities were calculated for the whole operation time of the reactors i.e. 75 days. ***Errors show standard deviation of triplicate measurements from the same reactor. Units are [1] (mL/gVS added), [2] (pgram COD/cell. d), [3](pmol CH_4/cell. d), [4] (mg/L) and [5] (mg/L).

Table 5. Correlation analysis of physiochemical parameters in digestates on day 75

Parameters	Methane yield	pH	Alkalinity	TVFA	NH_3-N
pH	.405				
Alkalinity	.670	.944*			
TVFA	.022	.447	.416		
NH_3-N	.187	.647	.632	.967**	
Al	.198	.324	.390	-.116	.042
As	.161	.214	.268	-.378	-.217
B	.331	.108	.263	-.238	-.103
Ba	.246	-.741	-.480	-.284	-.384
Ca	.148	-.844	-.623	-.459	-.578
Cd	.169	-.726	-.494	-.512	-.571
Co	.400	.603	.664	.923*	.964**
Cr	.122	.153	.219	-.302	-.165
Cu	-.079	.708	.517	.655	.695
Fe	.539	.644	.745	.850	.924*
K	-.008	-.833	-.645	-.541	-.637
Mg	.315	.033	.193	-.324	-.197
Mn	.945*	.399	.633	.193	.314
Mo	.330	.340	.422	-.343	-.149
Na	-.239	-.865	-.746	-.536	-.653
Ni	.488	-.013	.182	-.580	-.429
Pb	.418	-.178	.064	-.279	-.204
Si	.299	.363	.305	-.110	-.038
Ti	-.064	.127	.132	-.293	-.177
V	.009	-.910*	-.730	-.462	-.610
Zn	.261	-.206	-.021	-.430	-.350
Ni+Co+Mn	.969**	.403	.650	.160	.293
Ni+Co	.679	.221	.453	-.261	-.085

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). Positive and negative correlations with significant are highlighted in dark green and dark red respectively. Lower and lowest positive and negative correlations are highlighted in lighter and lightest green and red respectively.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LCO
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LCBW
LBA
LIBA
(B)
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Operating time (days)
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LCO
DIAB
LCBW
LBA
LIBA
(C)
Total alkalinity (g/L)
Operating time (days)
LFA
DIBA
LCBW
LBA
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(D)
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Operating time (days)
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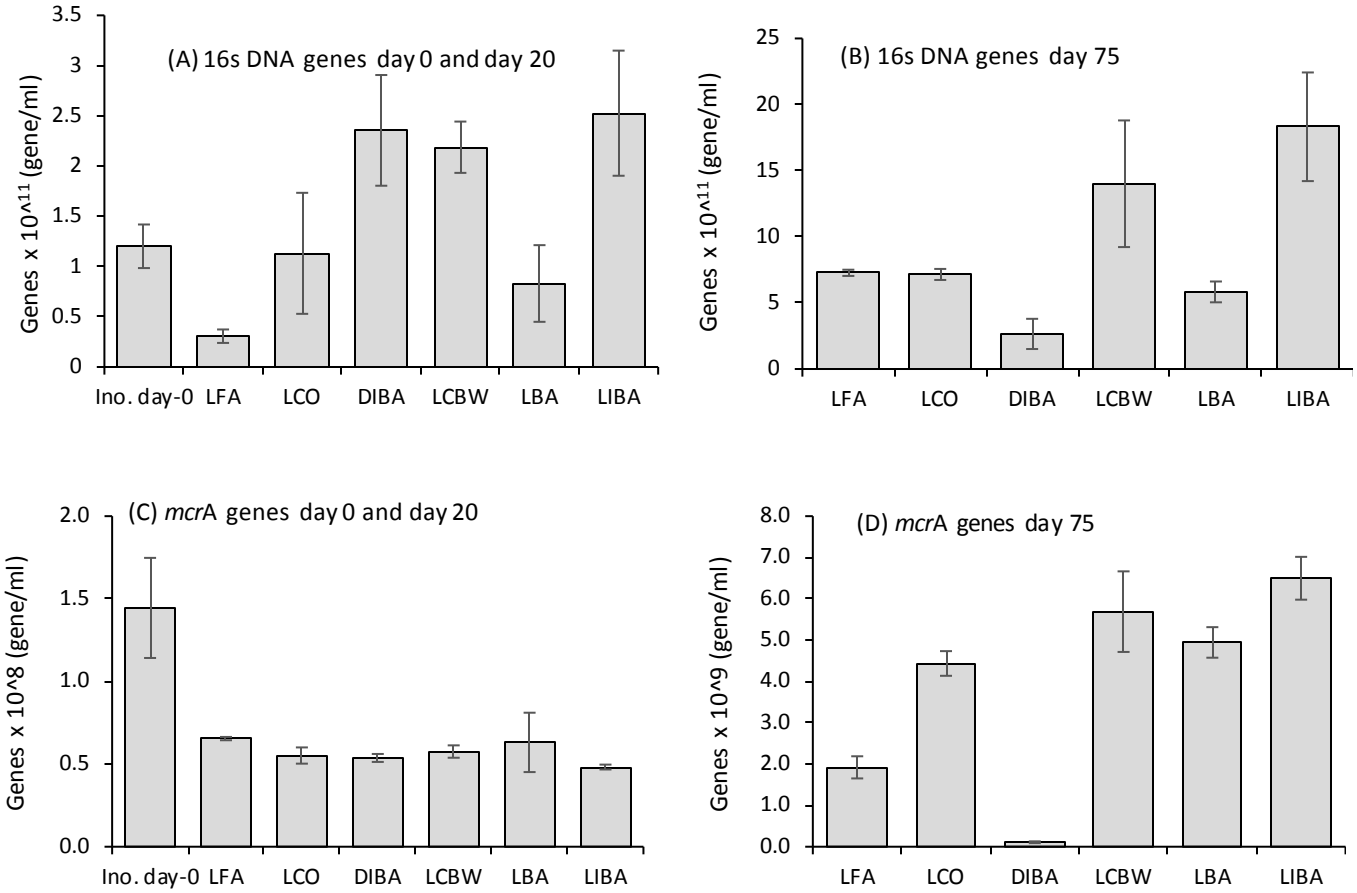
LBA
LIBA
LCO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1.5
1
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0
Ino. day-0
LFA
LCO
DIBA
LCBW
LBA
LIBA
(B) 16s DNA genes day 75
Genes x 10^11 (gene/ml)
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20
15
10
5
0
LFA
LCO
DIBA
LCBW
LBA
LIBA
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Genes x 10^8 (gene/ml)
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1.5
1.0
0.5
0.0
Ino. day-0
LFA
LCO
DIBA
LCBW
LBA
LIBA
(D) mcrA genes day 75
Genes x 10^9 (gene/ml)
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7.0
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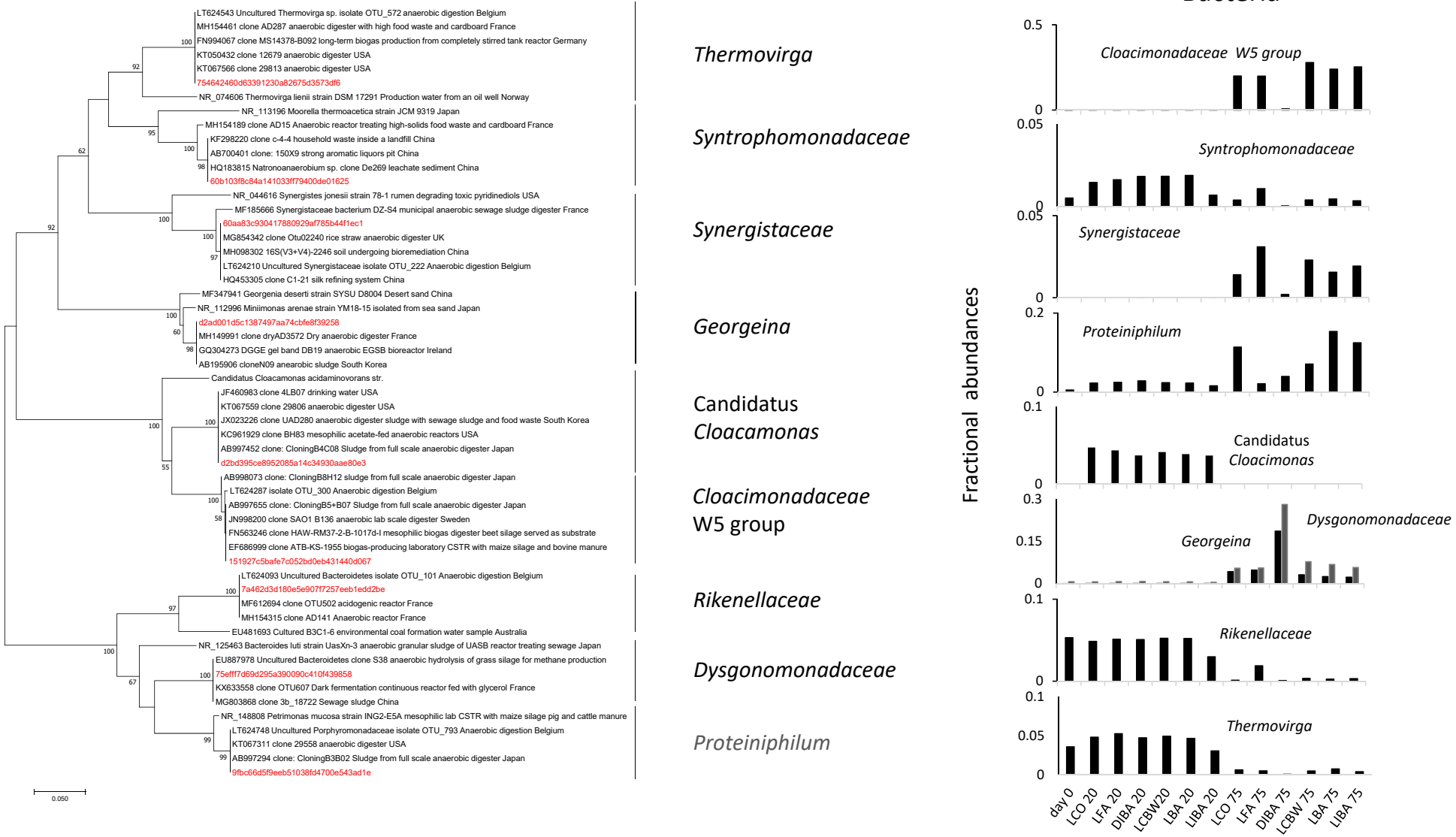
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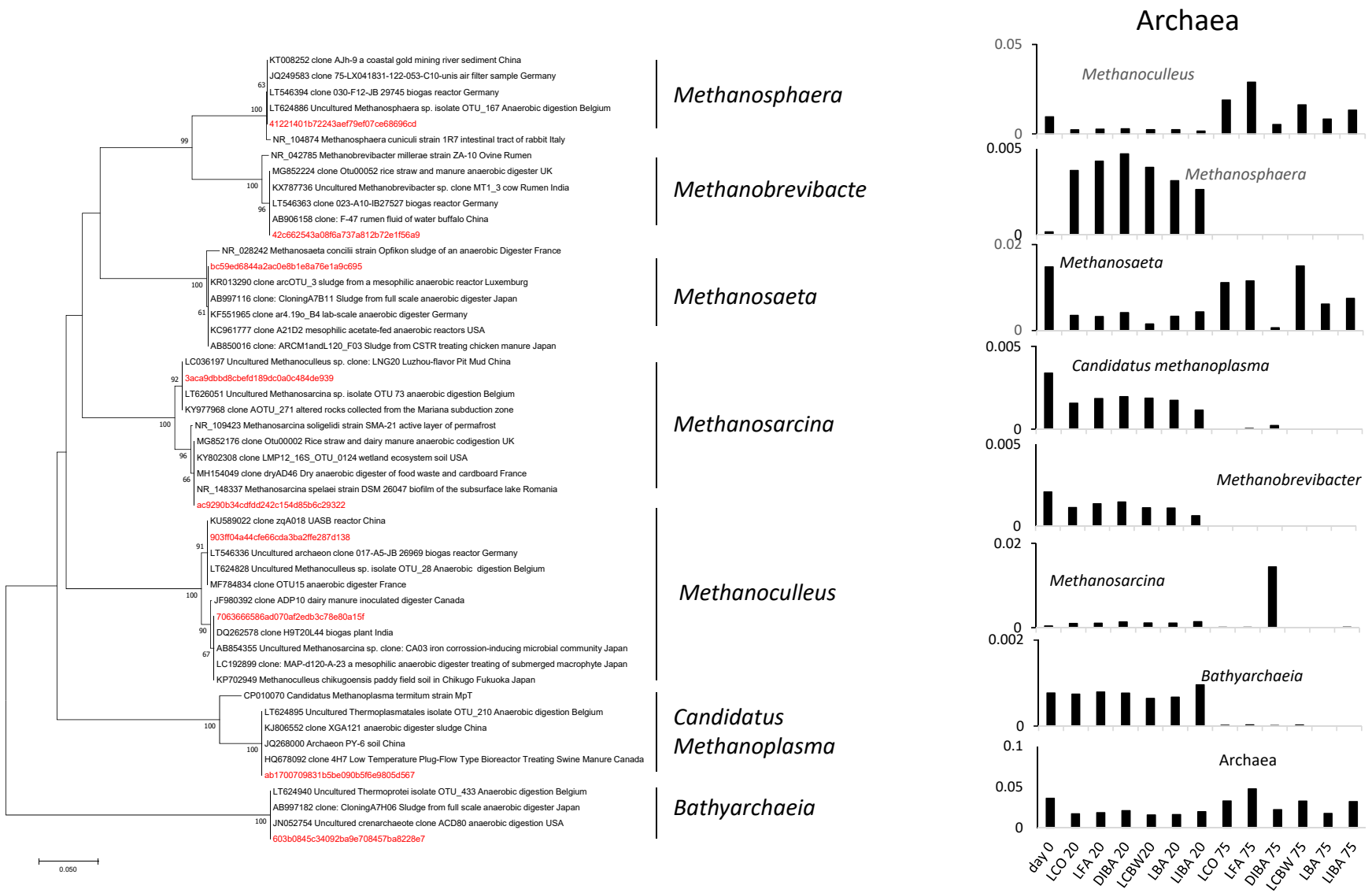
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KX787736 Uncultured Methanobrevibacter sp. clone MT1_3 cow Rumen India
LT546363 clone 023-A10-IB27527 biogas reactor Germany
AB906158 clone: F-47 rumen fluid of water buffalo China
42c662543a08f6a737a812b72e1f56a9
NR_028242 Methanosaeta concilii strain Opfikon sludge of an anaerobic Digester France
bc59ed6844a2ac0e8b1e8a76e1a9c695
KR013290 clone arcOTU_3 sludge from a mesophilic anaerobic reactor Luxemburg
AB997116 clone: CloningA7B11 Sludge from full scale anaerobic digester Japan
KF551965 clone ar4.19o_B4 lab-scale anaerobic digester Germany
KC961777 clone A21D2 mesophilic acetate-fed anaerobic reactors USA
AB850016 clone: ARCM1andL120_F03 Sludge from CSTR treating chicken manure Japan
LC036197 Uncultured Methanoculleus sp. clone: LNG20 Luzhou-flavor Pit Mud China
3aca9dbbd8cbefd189dc0a0c484de939
LT626051 Uncultured Methanosarcina sp. isolate OTU 73 anaerobic digestion Belgium
KY977968 clone AOTU_271 altered rocks collected from the Mariana subduction zone
NR_109423 Methanosarcina soligelidi strain SMA-21 active layer of permafrost
MG852176 clone Otu00002 Rice straw and dairy manure anaerobic codigestion UK
KY802308 clone LMP12_16S_OTU_0124 wetland ecosystem soil USA
MH154049 clone dryAD46 Dry anaerobic digester of food waste and cardboard France
NR_148337 Methanosarcina spelaei strain DSM 26047 biofilm of the subsurface lake Romania
ac9290b34cdfdd242c154d85b6c29322
KU589022 clone zqA018 UASB reactor China
903ff04a44cfe66cda3ba2ffe287d138
LT546336 Uncultured archaeon clone 017-A5-JB 26969 biogas reactor Germany
LT624828 Uncultured Methanoculleus sp. isolate OTU_28 Anaerobic digestion Belgium
MF784834 clone OTU15 anaerobic digester France
JF980392 clone ADP10 dairy manure inoculated digester Canada
7063666586ad070af2edb3c78e80a15f
DQ262578 clone H9T20L44 biogas plant India
AB854355 Uncultured Methanosarcina sp. clone: CA03 iron corrossion-inducing microbial community Japan
LC192899 clone: MAP-d120-A-23 a mesophilic anaerobic digester treating of submerged macrophyte Japan
KP702949 Methanoculleus chikugoensis paddy field soil in Chikugo Fukuoka Japan
CP010070 Candidatus Methanoplasma termitum strain MpT
LT624895 Uncultured Thermoplasmatales isolate OTU_210 Anaerobic digestion Belgium
KJ806552 clone XGA121 anaerobic digester sludge China
JQ268000 Archaeon PY-6 soil China
HQ678092 clone 4H7 Low Temperature Plug-Flow Type Bioreactor Treating Swine Manure Canada
ab1700709831b5be090b5f6e9805d567
LT624940 Uncultured Thermoprotei isolate OTU_433 Anaerobic digestion Belgium
AB997182 clone: CloningA7H06 Sludge from full scale anaerobic digester Japan
JN052754 Uncultured crenarchaeote clone ACD80 anaerobic digestion USA
603b0845c34092ba9e708457ba8228e7
0.050
Methanosphaera
Methanobrevibacte
Methanosaeta
Methanosarcina
Methanoculleus
Candidatus Methanoplasma
Bathyarchaeia
Archaea
Methanoculleus
Methanosphaera
Methanosaeta
Candidatus methanoplasma
Methanobrevibacter
Methanosarcina
Bathyarchaeia
Archaea
day 0
LCO 20
LFA 20
DIBA 20
LCBW20
LBA 20
LIBA 20
LCO 75
LFA 75
DIBA 75
LCBW 75
LBA 75
LIBA 75