



# Characterization of Core Microbiomes and Functional Profiles of Mesophilic Anaerobic Digesters Fed With *Chlorella vulgaris* Green Microalgae and Maize Silage

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Microalgal biomass is an alternative feedstock for biogas production although its C/N ratio is usually lower than optimal, therefore co-fermentation is recommended. Biogas production from photoautotrophically grown *Chlorella vulgaris* (*C. vulgaris*) biomass (240 mL CH<sub>4</sub> g oTS<sup>-1</sup>) and co-fermentation with maize silage (330 mL CH<sub>4</sub> g oTS<sup>-1</sup>) has been studied in semi continuous laboratory biogas fermenters. Maize silage control yielded 310 mL CH<sub>4</sub> g oTS<sup>-1</sup>. The microbial community and the read-based functional profiles, derived from these data, were examined during the process by using next-generation metagenome Ion Torrent sequencing technology. The read-based core microbiome consisted of 92 genera from which 60 abundant taxa were directly associated with the microbial methane producing food chain. The data-set was also analyzed in a genome-based approach. Sixty-five bins were assembled, 52 of them belonged in the core biogas producing genera identified by the read-based metagenomes. The read-based and genome-based approaches complemented and verified each other. The functional profiles indicated a variety of glycoside hydrolases. Substantial rearrangements of the methanogen functions have also been observed. Co-fermentation of algal biomass and plant biomass can be carried out for an extended period of time without process failure. The microbial members of the inoculum are well-conserved, feedstock composition changes caused mostly relative abundance alterations in the core microbiome.

**Keywords:** biogas, microalgae, metagenome, core microbiome, functional characteristics, binning, co-fermentation

## INTRODUCTION

Biomass utilization for alternative energy recovery is commonly considered to be a major contribution to the renewable energy production targets (Holm-Nielsen et al., 2009; Rehl and Müller, 2011; Mao et al., 2015; Hijazi et al., 2016). Primary biomass, produced by photosynthesis, currently comprise predominantly terrestrial plants (Gunaseelan, 1997; Schenk et al., 2008).

Microalgae may represent an alternative to terrestrial crops, because they have higher photosynthetic efficiency and faster growth rates, and they can be cultivated in various aqueous media, like sea, brackish water, or wastewaters (Posten and Schaub, 2009; Debowski et al., 2013; Posadas et al., 2014; Guldhe et al., 2017; Chen et al., 2018). Benefits of a microalgal biomass include versatility of utilization for energy purposes such as biohydrogen, bioethanol, biodiesel, and biogas production (Posten and Schaub, 2009; Harun et al., 2010; Klassen et al., 2016; Uggetti et al., 2016), and complex biorefinery applications (Yen et al., 2013). The main technological disadvantages, i.e., requirement for large land area and efficient harvesting, are addressed in ongoing research (Estime et al., 2017; Ummalyma et al., 2017).

Microalgal biomass is a promising feedstock for anaerobic digestion (AD), as it is usually rich in lipids, carbohydrates, and proteins, and does not contain recalcitrant lignin (Yen et al., 2013; Ward et al., 2014). The generated biogas yields are typically 10–20% lower, but the methane content is 7–13% higher than that from maize silage (Mussgnug et al., 2010). Successful AD of algal biomass depends on the efficient deconstruction of the cell wall (Córdova et al., 2018). Physical, mechanical, chemical, and thermal pre-treatment methods have been tested to enhance digestion efficiency (Alzate et al., 2012; Lam and Lee, 2012; Passos et al., 2013, 2014, 2018; Lavrič et al., 2017). These methods could improve biogas yield, but the energy input is quite high (Carrere et al., 2016). Hydrolytic enzymes have also been proven effective in microalgal biomass pre-treatment (Demuez et al., 2015; Mahdy et al., 2015), although the economy of the process is frequently jeopardized (Vergara-Fernández et al., 2008). Another tool to make the microalgal biomass a suitable AD feedstock is to adjust the carbon to nitrogen ratio (C/N) to a range between 20 and 30 (Yadvika et al., 2004). Low C/N ratios, i.e., <10–15, which are characteristic of microalgal biomass, result in increased levels of free ammonia, i.e., >0.15 g NH<sub>3</sub>-N L<sup>-1</sup> or >3–4 g NH<sub>4</sub><sup>+</sup> L<sup>-1</sup>, that may become inhibitory (Khalid et al., 2011; Kwietniewska and Tys, 2014). Co-digestion is a straightforward strategy to overcome this obstacle (Mahdy et al., 2015; Wirth et al., 2015a,b; Rétfalvi et al., 2016; Wang W. et al., 2016).

The microbiological events taking place in the AD reactors fed with microalgae are not fully understood. Early studies used Denaturing Gradient Gel Electrophoresis (DGGE) and identified *Methanosaeta* sp. and acetoclastic methanogenesis as the main route to biogas formation (Lakaniemi et al., 2011; Zamalloa et al., 2012). The same conclusion was reached by analyzing the methyl-coenzyme M reductase  $\alpha$ -subunit gene (*mcrA*) by high-throughput amplicon sequencing (Ellis et al., 2012). Amplicon sequencing has limitations arising from possible PCR bias, choice of primers, variable copy numbers in bacterial genomes, sequence variation in closely related taxa, and lack of information about the functions of microbes (Becker et al., 2000; Větrovský and Baldrian, 2013; Oulas et al., 2015; Campanaro et al., 2018b). Development of high-throughput molecular tools and bioinformatics allowed the investigation of total DNA and could provide a more detailed picture of the microbiome. Ion Torrent PGM<sup>TM</sup> was used to explore the microbial composition of *Spirulina* AD at alkaline conditions (Nolla-Ardèvol et al., 2015b). A broad range of active membrane proteins were

identified, which were responsible for starch degradation, sugar uptake, metabolism of peptides and osmoprotectants. Bacterial community alterations were observed during the co-digestion of microalgae with used cooking oil, mill residue, and maize silage (Rétfalvi et al., 2016). Similar changes were reported in the AD of *Scenedesmus obliquus* and microalga-bacteria mix (Wirth et al., 2015a,b). Co-digestion of algal biomass with terrestrial plant biomass apparently facilitated the hydrolytic step.

Search for core microbiota, i.e., the microbial community present throughout the time span of the process and independently of the feeding regime, has gained increasing attention in studying complex systems in microbial ecology (Nagai et al., 2010; Xia et al., 2011; Jami and Mizrahi, 2012; Schären et al., 2017). Core Operational Taxonomic Units (OTUs), based on 16S rRNA gene amplicon libraries have been reported as being present in diverse anaerobic communities (Rivière et al., 2009; Huse et al., 2012; Rui et al., 2015; Calusinska et al., 2018). Core microbial communities can be more precisely identified by the combination of read-based and binning metagenome techniques (Nolla-Ardèvol et al., 2015a; Campanaro et al., 2016, 2018a; Heintz-Buschart et al., 2016; Stolze et al., 2016; Treu et al., 2016; Zhu et al., 2018). Metagenomics studies also allow for the extrapolation to ecological functional networks (Schlüter et al., 2008; Wirth et al., 2012; Campanaro et al., 2016; Stolze et al., 2016), although this is of limited information, because the abundances of genes coding for enzymes do not necessarily reflect biological activity (De Vrieze et al., 2016).

We have demonstrated earlier (Wirth et al., 2015a,b) that an algal-bacterial mixed biomass is a suitable substrate for biogas generation. In the present study we report on biogas production from photoautotrophically grown *Chlorella vulgaris* microalgal biomass. Although microbiologically pure algal biomass is certainly more expensive biogas substrate than an algal-bacterial mixture, it was selected for this study so that the bacterial components, other than the constituents of the microalgal phycosphere, would not distort the core AD microbiome. To our best knowledge, there are no data about the core microbiota digesting microalgal biomass in semi Continuously Stirred Tank Reactor (CSTR) type laboratory biogas fermenters. Therefore, our aim was to determine the response of the core biogas producing microbial community in time to the feedstock, i.e., *Chlorella* algal biomass. The microbial community and the metagenomics-based functional profile, derived from these data, were monitored during the process by using high-throughput sequencing technology (Ion Torrent PGM<sup>TM</sup>). The AD parameters and microbial communities in the anaerobic reactors fed with *C. vulgaris*, maize silage (internal control in the experiment), or the mixture of the two feedstocks were compared.

## MATERIALS AND METHODS

### Feedstock Composition

Biomass of *C. vulgaris* was obtained from Lisi (Xi'an) Biotech INC (Shaanxi, Xi'an, China). *C. vulgaris* had been cultivated in photobioreactors, under natural light illumination at ambient temperature. The protein content was 58.50% and the bacterial count was <100,000 cfu g<sup>-1</sup>, according to the specifications of

**TABLE 1** | Characterization of the feedstocks used in the experiment.

Substrate	Wet mass N (mg/g)	Wet mass C (mg/g)	C/N ratio	TS%	oTS%
Maize silage	5.25	198.75	45.25/1	32.27	91.79
<i>C. vulgaris</i>	8.55	45.22	5.28/1	91.71	92.87
Co-digestion	6.72	107.27	15.96/1	71.98	90.54

N, nitrogen content; C, carbon content; TS, total solid content; oTS, organic total solid content.

the producer. We have confirmed the cfu value by plating the lyophilized material on complex medium (Luria-Bertani agar). The biomass was stored at 4°C until utilization.

Maize silage was obtained from the mesophilic biogas facility “Zöldforrás Ltd.,” located near the city of Szeged, Hungary. The inoculum sludge was originated from the same place. The plant uses pig slurry and ensilaged plant biomass (maize silage and sweet sorghum mixture) as feedstock. The main parameters of the microalgal biomass and maize silage feedstocks are presented in **Table 1**.

## Anaerobic Digestions

Anaerobic digestions were carried out in three of 5 L CSTRs (Kovács K. L. et al., 2013) in fed-batch operational mode. The experimental design and time course followed the scheme described previously (Wirth et al., 2015a,b). In short, the apparatus can be fed continuously or intermittently via a piston-type delivery system, the fermentation effluent is removed through an air-tight overflow. The reactors are equipped with a spiral strip mixing device driven by an electronic engine. An electronically heated jacket surrounds the cylindrical stainless steel body, electrodes for the measurement of pH and redox potential are inserted through the reactor wall, in sealed sockets. The device can be drained at the bottom. The evolved gas leaves the fermentor through the top plate, where ports for gas sampling and the delivery of liquids by means of syringes through silicone rubber septa are also installed. Gas volumes are measured with thermal mass flow devices (DMFC SLA5860S, Brooks). Following the 1 month “start-up” incubation the three reactors were fed as follows: one fermentor received *C. vulgaris* biomass at a loading rate of 1 g oDM L<sup>-1</sup> day<sup>-1</sup> (oDM = organic dry matter), one reactor was supplied with a mixture of *C. vulgaris* + maize silage (0.5 g oDM L<sup>-1</sup> day<sup>-1</sup> each) and the third one with maize silage (1 g oDM L<sup>-1</sup> day<sup>-1</sup>). After the “start-up” phase the accumulated gas volume was recorded in every 4 h. The composition of the evolved biogas was measured with a gas chromatograph (6890N Network GC System, Agilent Technologies) equipped with a 5Å molecular sieve column (length 30 m, I.D. 0.53 megabore, film 25 µm). Ultrapure Ar was used as carrier gas, the detection limit for CH<sub>4</sub> and CO<sub>2</sub> was about 30 ppm. The determination of digestion parameters, i.e., organic dry matter, C to N ratio, NH<sub>4</sub><sup>+</sup>-N and volatile organic acids/total inorganic carbon was done as previously described (Wirth et al., 2015a,b).

## Organic Acid Analysis

Samples for organic acid analysis were taken from the liquid phase of the reactors. The samples were centrifuged

(16,000 g for 10 min,) and the supernatant was filtered through polyethersulfone (PES) centrifugal filter (PES 516-0228, VWR) at 16,000 g for 20 min. The concentrations of volatile organic acids were determined with HPLC (Hitachi LaChrome Elite) equipped with refractive index detector L2490. The separation was performed on an ICsep ICE-COREGEL–64H column. The temperature of the column and detector was 50 and 41°C, respectively. The eluent was 0.01 M H<sub>2</sub>SO<sub>4</sub> (0.8 mL min<sup>-1</sup>). Acetate, propionate and butyrate were determined (detection range 0.01–10 g L<sup>-1</sup>), the latter two were present in traces relative to acetate and therefore they are not included in the results.

## DNA Isolation for Metagenomics Study

The composition of the microbial community was investigated six times during the experimental period, i.e., at the starting point and at the end of weeks 1, 5, 9, 12, and 16 ( $n = 16$ ). For total community DNA isolation 2 mL of samples were used. DNA extractions were carried out using a slightly modified version of the Zymo Research Fecal DNA kit (D6010, Zymo Research, Irvine, USA). The lysis mixture contained 100 µL of 10% CTAB (cetyltrimethylammonium bromide) to improve the efficiency (Wirth et al., 2015a,b). After lysis (bead beating was performed by Vortex Genie 2, bead size: 0.1 mm; beating time: 15 min, beating speed: max), the Zymo Research kit protocol was followed. The quantity of DNA was determined in a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA) and a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, USA). DNA purity was tested by agarose gel electrophoresis and on an Agilent 2200 TapeStation instrument (Agilent Technologies, Santa Clara, USA).

## Next Generation Sequencing and Data Handling

The recommendations of the Ion Torrent PGM™ sequencing platform were closely followed (Life Technologies, USA). The preparation of sample libraries was made by Life Technologies IonXpress fragment plus library protocol (4471269), Ion device library quantitation kit (4468802) and Step One Real Time PCR (Applied Biosystems) were used to quantify the samples. The emulsion was done with OneTouch 2 and Ion OneTouch ES devices by using the Ion PGM Template OT2 200 kit (4480974). Barcoding was carried out by IonXpress barcode kit (4471250). Sequencing was performed with Ion PGM 200 Sequencing kit (4474004) on Ion Torrent PGM 316 chip. The characteristic fragment parameters are summarized in **Supplementary Table 1**. The sequence reads covered the metagenomes sufficiently as demonstrated by the rarefaction curves (**Supplementary Figure 1**).

The primary sequences produced by Ion Torrent PGM™ were further analyzed by the Diamond software, applying the default LCA (Lowest Common Ancestor) algorithm (Buchfink et al., 2014). Diamond filtering parameters were set as follows: Blast Mode: BlastX, Min Score: 50, Max Expected: 0,01, Top Percent: 10, Min Support: 1. NCBI nr and EMBL-EBI InterPro databases were used for taxonomic and functional alignment (Federhen,

2015). The data were analyzed by Megan6 (Huson et al., 2007, 2011, 2016).

## Availability of Data and Material

Sequences are available on MG-RAST under the study name: “Anaerobic digestion of *C. vulgaris* microalgal biomass” (ID: mgp20184). Data are also available on NCBI Sequence Read Archive (SRA) under the submission number: PRJNA544905.

## Statistical Analysis of Metagenomics Data

Megan6 (version 6.8.18) was used to investigate microbial community and export data for statistical calculations. UPGMA (Unweighted Pairwise Grouping Method with Arithmetic mean) with Bray-Curtis method was employed to cluster the samples (Bray and Curtis, 1957).

For microbial and functional core calculation the interactive web tool MetaCoMET (Metagenomics Core Microbiome Exploration Tool) was used (Biom formatted data were exported by Megan6). Default parameter sets were fixed at absolute read count threshold: 100, with the persistence Venn diagram type (additional settings: between group relative abundance: 0.8, samples in group relative: 0.8) (Wang Y. et al., 2016). The functional profiles of the reactors, fed with the various feedstocks, were investigated using metagenomics data and EMBL-EBI InterPro database (Schlüter et al., 2008; Wirth et al., 2012; Campanaro et al., 2016, 2018a; Stolze et al., 2016; Finn et al., 2017; Maus et al., 2017).

Statistical Analysis of Metagenomic Profiles (STAMP; version 2.1.3 Parks and Beiko, 2010; Cai et al., 2016) was used to compute the abundance differences in the case of whole microbiome and core taxa (<http://kiwi.cs.dal.ca/Software/STAMP>). Dissimilar taxa were identified with two-sided White's non-parametric *t*-test (White et al., 2009) at 0.95 confidence intervals and the results with *q*-value (corrected *p*-value) of <0.05 were retained.

## Metagenomic Binning

Galaxy Europe server was employed to pre-process the raw reads, i.e.: sequence filtering, mapping, quality checking (<https://usegalaxy.org/>) (Goecks et al., 2010; Afgan et al., 2016). Low-quality reads were filtered by Prinseq (minimum length: 60; minimum score: 15; quality score threshold to trim positions: 20; sliding window used to calculate quality score: 1) (Finn et al., 2011). Following quality check with FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) (Supplementary Table 4) the good quality reads were co-assembled with Megahit (minimum contig length: 2500; minimum k-mer size: 21; maximum k-mer size: 141) (Li et al., 2015). After simplifying the header of contig FASTA file using the Anvi'o script “reformat-fasta,” Bowtie2 was employed to map back the filtered reads to the contigs (Langmead and Salzberg, 2012). Then we used Anvi'o V5, following the default “metagenomics” workflow (Eren et al., 2015). Briefly, in the first step a contig database was generated, where open reading frames were identified by Prodigal and each contig k-mer frequencies were computed (Hyatt et al., 2010). Then Hidden Markov Model (HMM) of single-copy genes were aligned by HMMER (Finn et al., 2011; Campbell et al., 2013;

Rinke et al., 2013; Simão et al., 2015) (Seemann T, <https://github.com/tseemann/barrnap>). We used InterProScan v5.31-70 on Pfam and Kaiju on NCBI nr database for functional and taxonomic annotation of contigs (Finn et al., 2014; Jones et al., 2014; Menzel et al., 2016; Agarwala et al., 2018). The outputs were imported into the contig database by using the “anvi-import” command. BAM files, made by Bowtie2, were employed for profiling contig database. This gave sample-specific information about the contigs, i.e., mean coverage. The sample-specific information was merged together using the “anvi-merge” command. Three automated binning programs, namely CONCOCT, METABAT 2, and MAXBIN 2, were employed to reconstruct microbial bins from the contigs (Alneberg et al., 2013; Kang et al., 2015; Wu et al., 2015). We also used the Anvi'o human-guided binning and interactive “anvi-refine” option (Delmont et al., 2018). The binning results were directed to the contig database by using “import-collection” command. Anvi'o interactive interface and “anvi-summarize” script was employed to visualize the data (Supplementary Table 4). The manual binning collection consisted of 65 bins accounting for 15,763,220 nucleotides, which represented 32% of all nucleotides stored in the profile database. Minimum Bin size was 8 kb. For average nucleotide identity (ANI) calculation the OrthoANIu algorithm was used (<https://www.ezbiocloud.net/tools/ani>) (Yoon et al., 2017). Figure finalization was made by open-source vector graphics editor Gimp 2.10.8 (<https://www.gimp.org/>).

## RESULTS AND DISCUSSION

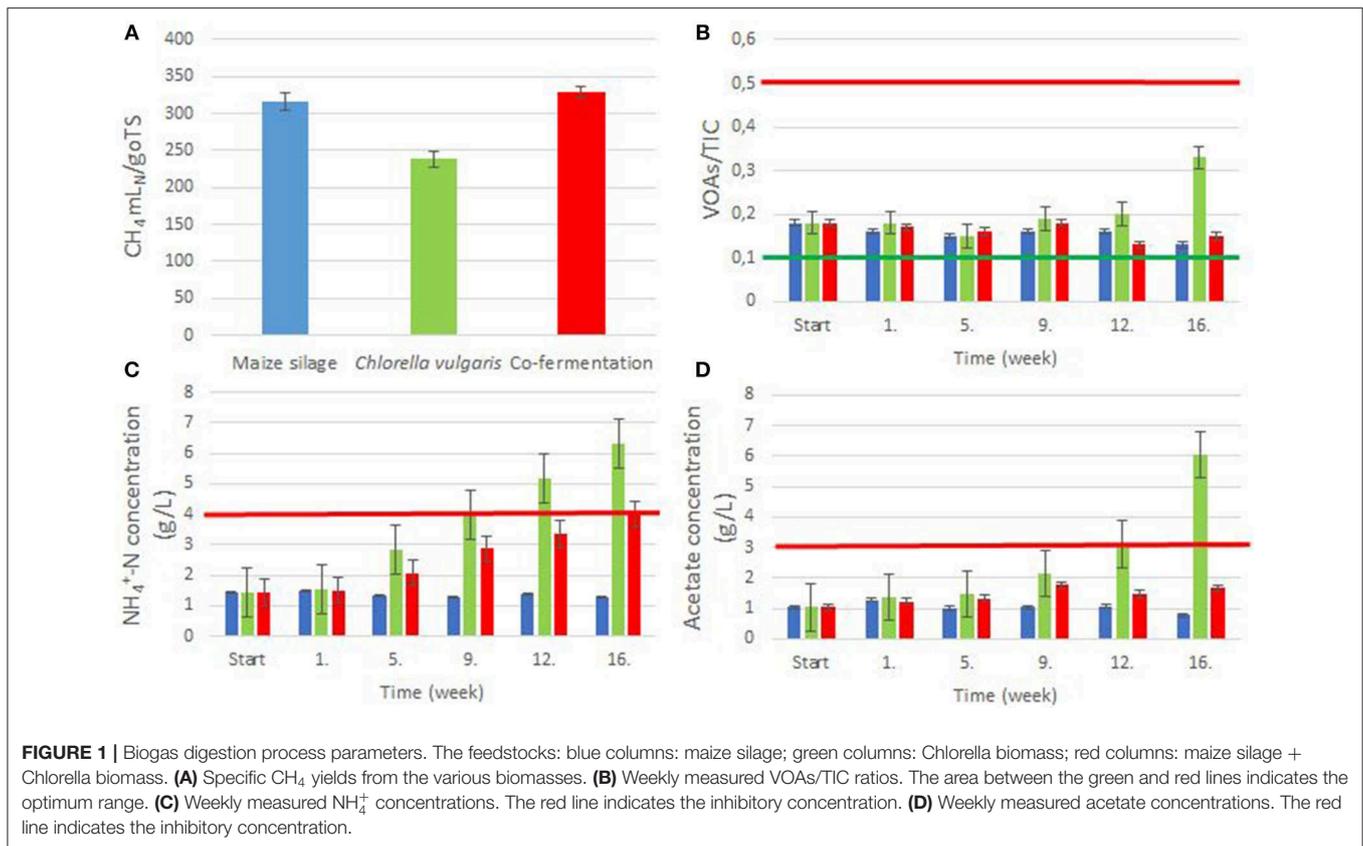
### Process Parameters During AD

During the anaerobic degradation of the various substrates, temperature ( $37 \pm 0.5^\circ\text{C}$ ), mixing speed ( $10 \text{ rpm min}^{-1}$ ) and pH (7.5–8.3) were continuously monitored by a software (Kovács K. L. et al., 2013). The redox potential was below  $-500 \text{ mV}$  throughout the experiment. By the end of the “start-up” period the residual biogas production ceased completely. Gas production data were collected during weeks 1 through 16 in every 4 h and the cumulative  $\text{CH}_4$  productions are plotted in Supplementary Figure 5. Figure 1A shows the  $\text{CH}_4$  yields.

The  $\text{CH}_4$  content in the evolved gas from *C. vulgaris* and from maize silage was 57–67 and 50–52%, respectively, these were comparable with previously reported data (Amon et al., 2007; Mussnug et al., 2010), although in the case of *C. vulgaris* we measured somewhat lower  $\text{CH}_4$  than in a previous study (Mussnug et al., 2010). Co-digestion of *C. vulgaris* and maize silage yielded an intermediate  $\text{CH}_4$  value of 57–59%.

The VOAs/TIC (Volatile Organic Acids/Total Inorganic Carbon) ratio is a reliable indicator of a stable digestion process (Lienen et al., 2013). The optimal ratio ranges between 0.1 and 0.5 (Leite et al., 2015). Most of our values fell within this range, due to the low organic loading rate (OLR), indicating a stable and balanced operation in all three reactors, although toward the end of the experimental period the VOAs/TIC in the reactor digesting *C. vulgaris* alone started to increase (Figure 1B).

The amount of  $\text{NH}_3\text{-N}$ , conveniently measured as  $\text{NH}_4^+\text{-N}$ , is also a critical indicator of stability of the biogas-forming process (Yenigün and Demirel, 2013). Levels above  $4 \text{ g NH}_4^+\text{-N L}^{-1}$ ,



corresponding to about  $0.15 \text{ g NH}_3\text{-N L}^{-1}$  under the current experimental conditions, apparently have a negative effect on the methanogenic community (Chen et al., 2008; Nielsen and Angelidaki, 2008). The inhibitory concentrations reported in earlier studies varied, depending on feedstock, inoculum, environmental conditions, and acclimation (Chen et al., 2008; Yenigün and Demirel, 2013; Gonzales-Fernandez et al., 2018). In the reactor fed with *C. vulgaris*, the  $\text{NH}_4^+\text{-N}$  content tended to increase, and by week 9, approached, and later exceeded the limiting inhibitory concentration (week 12:  $5.16 \text{ g NH}_4^+\text{-N L}^{-1}$  or  $0.26 \text{ g NH}_3\text{-N L}^{-1}$ ) (Figure 1C and Supplementary Figure 3). It is noteworthy that co-digestion showed balanced digestion and approached the limiting  $\text{NH}_4^+\text{-N}$  values ( $3.99 \text{ g NH}_4^+\text{-N L}^{-1}$  or  $0.15 \text{ g NH}_3\text{-N L}^{-1}$ ) only in week 16. The acetate concentrations (Figure 1D) exhibited a tendency similar to that of VOAs/TIC (Figure 1B). The acetate concentration in the *C. vulgaris* reactor reached the inhibitory limit of  $3 \text{ g L}^{-1}$  (Siegert and Banks, 2005) by week 12. Ammonia and acetate accumulation was due to the low C/N ratio of the microalgal biomass, although the pH remained stable in the *C. vulgaris* reactor because of the high TIC.

The hierarchical clustering indicated good correlation with ammonia and acetate concentrations suggesting that these parameters prominently affected the development of the microbial communities (Supplementary Figure 3). Co-digestion efficiently prevented ammonia and acetate accumulation corroborating earlier observations (Yen and Brune, 2007; Wirth et al., 2015a,b).

## The Core Microbial Community

Although we detected substantial alterations in the AD microbial communities fed with the various feedstocks, it is intriguing to see which members of the inoculum community persisted throughout the time span of the experiment in spite of the distinct feeding regimes. A mixed algal-bacterial biomass is the obvious choice of microalgal biomass production for large-scale biogas generation due to trivial economic feasibility reasons. It is, however, difficult to separate the bacterial community introduced with a mixed algal-bacterial biomass from the community introduced with the inoculum and adapted to this particular substrate. That is why in this study we used microbiologically pure *C. vulgaris* biomass substrate to properly establish the core community participating in the process. The core microbiome exploration tool was employed (MetaCoMET) in search for this core microbial community (Rivière et al., 2009; Rui et al., 2015). In the read-based metagenomics database the “genus” taxonomic rank was chosen to identify the key players because the depth of the sequencing allowed the taxonomic resolution at this level (Supplementary Figure 1). In addition to the read-based evaluation, we analyzed the same sequencing data-set by three binning programs to determine the genome-based taxa involved in the biogas forming process. Due to the high complexity of the AD microbial community and the drawbacks of short-read sequencing technology, only a limited number of taxa can be recovered by binning. The read-based and genome-centric

approaches are complementary in providing a more reliable picture of the AD microbial community.

We identified first the sub-cores, i.e., the genera present above threshold abundance (0.8%) at all 5 time points (and in the inoculum) in the reactor fed with each feedstock (**Figure 2A**). This distinguished 125 genera in the maize silage AD (31% of all genera detected in this reactor), 106 genera in the *C. vulgaris* AD (26% of all microbes in this reactor), and 113 genera in the co-digestion reactor (23% of the total microbes in this reactor).

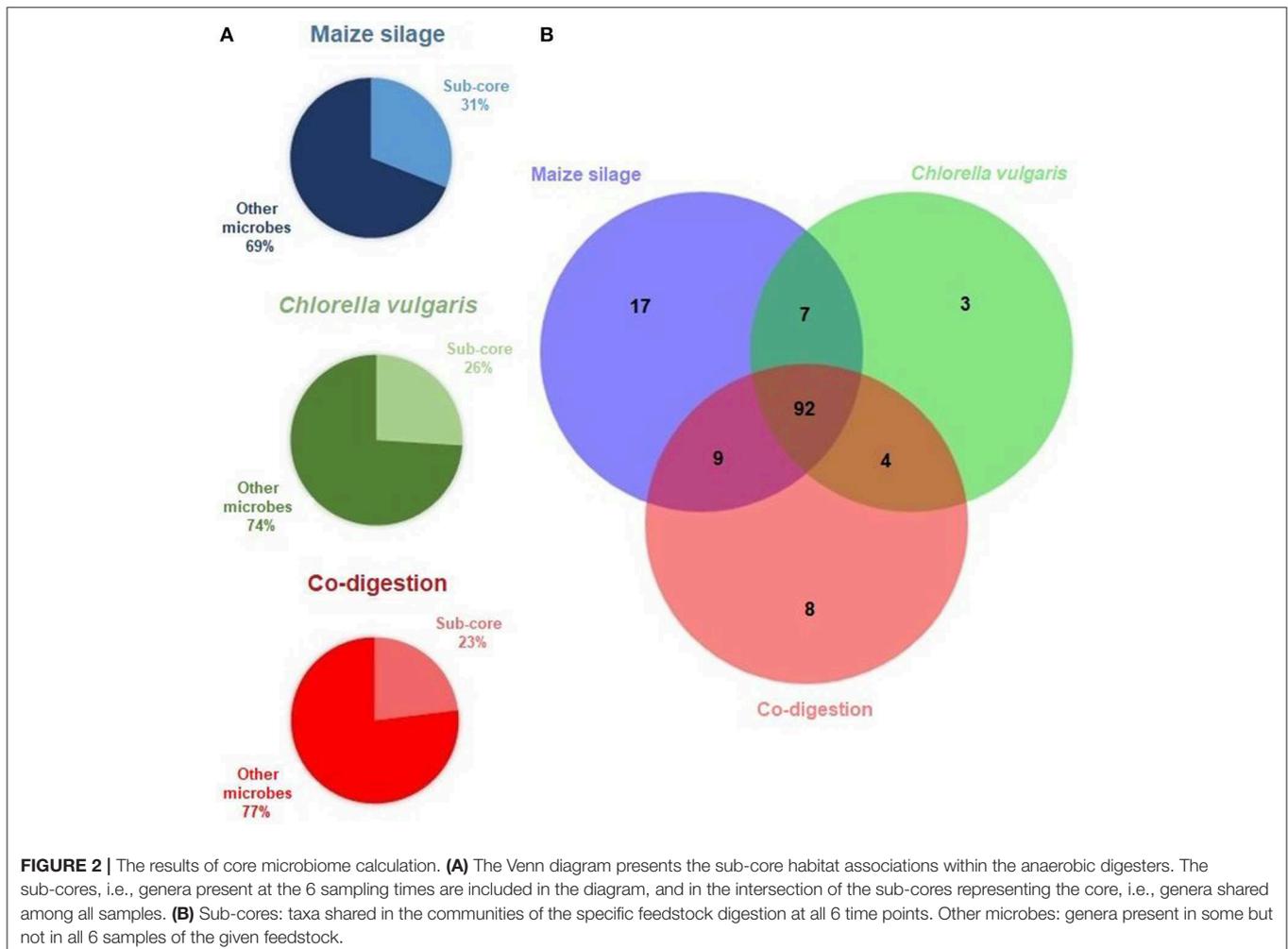
The core community overlapping in all reactors, i.e., the collection of genera found in all 16 metagenomes above threshold, comprised differently abundant 92 genera (**Figure 2B** and **Supplementary Table 2**). The composition of the core microbiome is shown in **Figure 3**. The high number of genera preserved in the core community suggests that the inoculum has a remarkable impact on the evolution of the microbial community in the AD reactor (De Vrieze et al., 2014; Han et al., 2016; Liu et al., 2017; Mahdy et al., 2017; Gonzales-Fernandez et al., 2018).

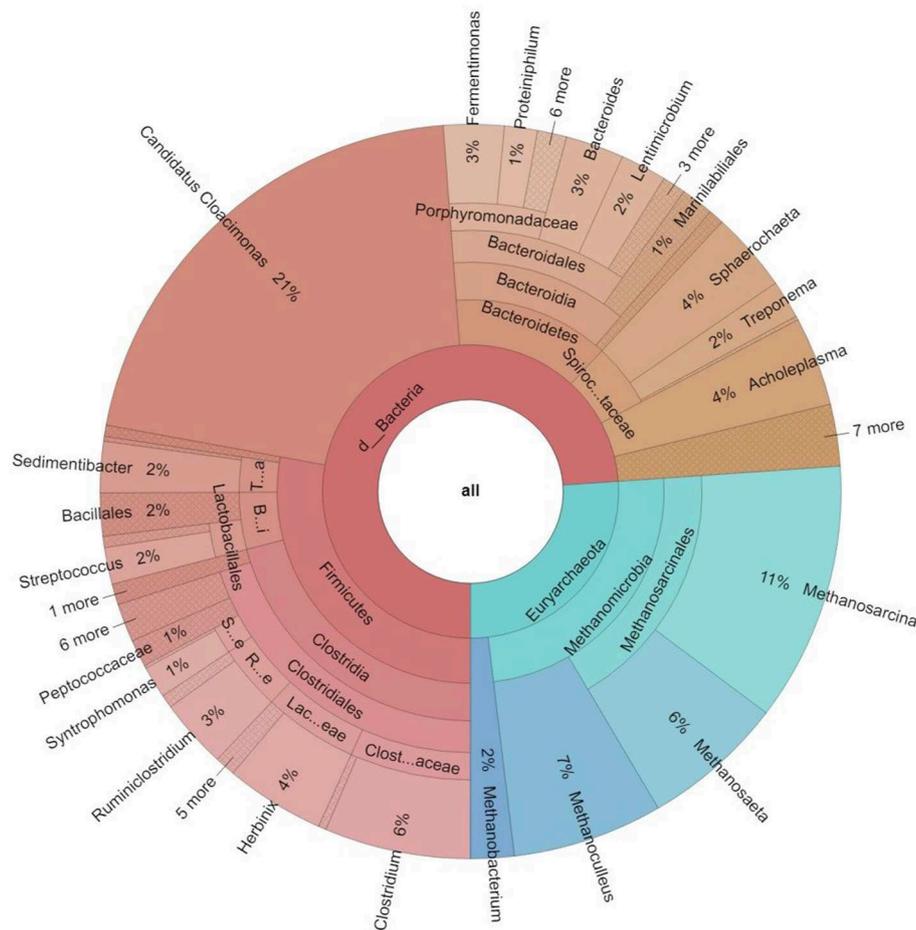
In the following discussion we combine the read-based metagenomics data with binning results (**Figure 4**). This

information was used to position the various core genera all along the AD process from polymer hydrolysis to biogas evolution. The assembly of the elements of the “core puzzle” is summarized in **Figure 5**. Sixty genera from the read-based core taxa could be associated with various stages of AD. From the 65 bins 52 could be related with the methane producing microbial food chain. It should be noted that 42 of these bins are listed in **Figure 5** because bins identified under the same name but distinguishable as separate bins are listed only once.

It general, it is noteworthy that the read-based sequence analyses are complemented adequately by the genome-based approach. The former established a good picture of the participating microbial community at genus level, while the latter indicated several species identification, but also numerous “uncultured” or partly “unknown” bins. This confirmed that the AD community is only partly understood in details (Campanaro et al., 2018a,b).

The core genera involved in polymer hydrolysis were searched for first (green box in **Figure 5**). Diversity characterized the order *Clostridiales*. This is in accordance with previous observations (Wirth et al., 2015a,b) (**Supplementary Figure 2**).





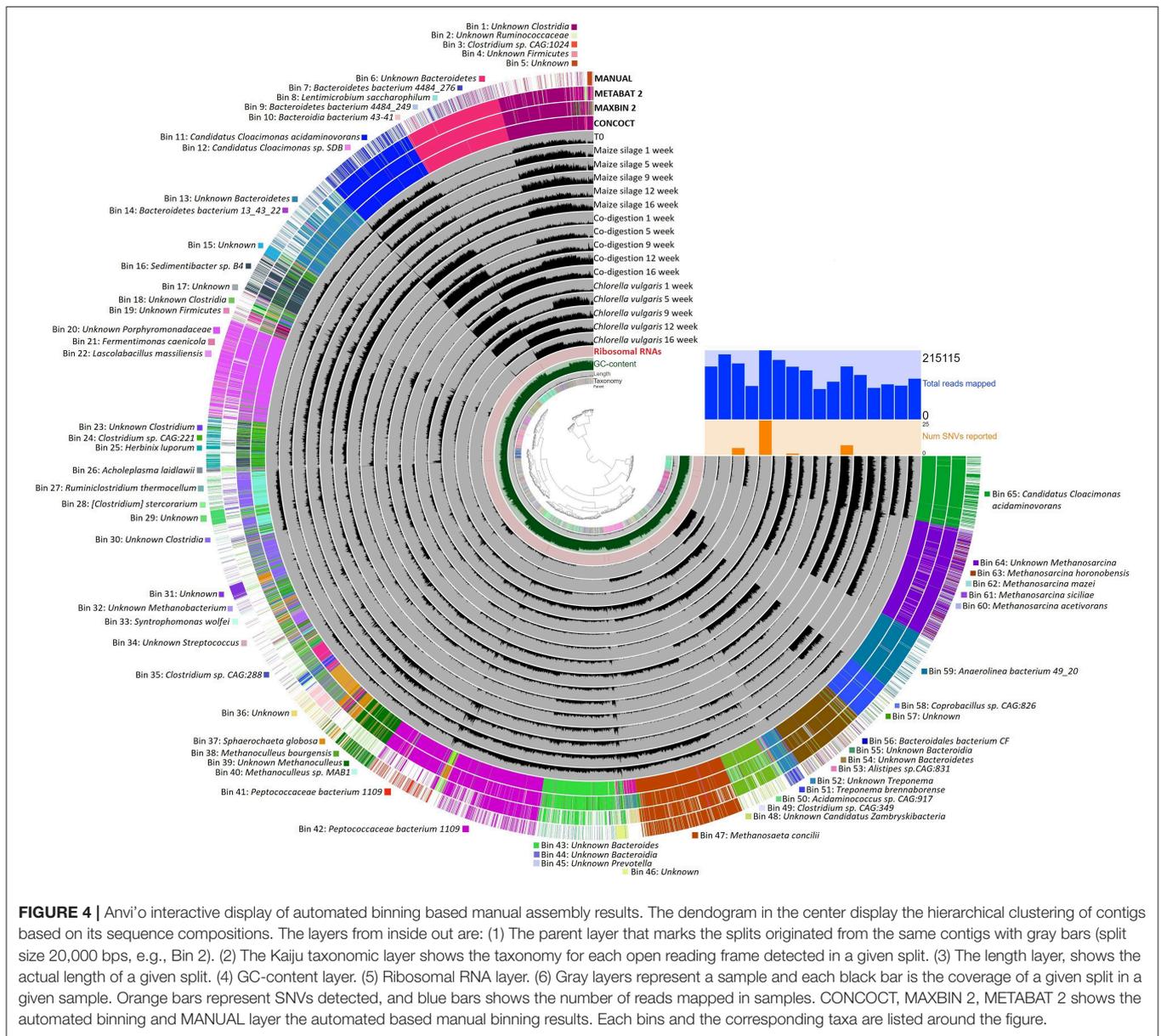
**FIGURE 3 |** Taxonomic distribution of the core microbiome, i.e., the taxa present in all 16 metagenomes at domain, phylum, class, order, family and genus levels. The % abundances indicate the mean averages of all samples investigated. Only the genera present in  $\geq 1\%$  abundance are listed on the outermost circle. In the domain *Bacteria* the genera *Candidatus Cloacimonas* and *Clostridium* prevailed, among *Archaea* *Methanosarcina*, *Methanoculleus*, and *Methanosaeta* predominated.

From the members of class *Clostridia* nine bins were detected: three *Unknown Clostridia* (Bin 1, Bin 18, and Bin 30), *Unknown Clostridium* (Bin 23), four *Clostridium* sp. (CAG:1024, CAG:221, CAG:288, CAG:349 as Bin 3, Bin 24, Bin 35, and Bin 49, respectively) and *Clostridium stercorarium* (Bin 28) (Figure 4). These bins contain a wide variety of putative glycoside hydrolases and genes coding for hydrogen production (Supplementary Table 4). The possible end products from polysaccharide degradation are volatile fatty acids (VFAs) (Lin et al., 2007; Wirth et al., 2012) (Figure 5).

The genome fragment of *Herbinix luporum* (Bin 25) is predicted to possess cellulose degrading activity, genes coding for endoglucanase and cellobiohydrolase (Koeck et al., 2015). The genera *Clostridia* and *Herbinix* showed the largest variances among *Bacteria* when the substrates maize silage and *C. vulgaris* biomasses were compared (Figure 6). These hydrolytic genera were considerably more abundant in the reactors supplied with maize silage and a similar trend was apparent in the co-digestion reactor.

The genus *Bacteroides* is known to possess hydrolytic properties (Pobeheim et al., 2010; Campanaro et al., 2016). Binning uncovered eleven *Bacteroidetes* genome fragments (Bins 6, 7, 9, 10, 13, 14, 43, 44, 54, 55, and 56) containing genes for hydrolyzing enzymes (Supplementary Table 4). The genera *Clostridium* and *Bacteroides* have been also implicated in the efficient deconstruction of microalgal cell wall (Yen and Brune, 2007; Wirth et al., 2015a).

*Ruminiclostridia* are able to degrade cellulose and hemicellulose, and ferment various sugars to VFAs (Ravachol et al., 2015). *Ruminiclostridium thermocellum* (Bin 27) and an *unknown Ruminococcaceae* (Bin 2) were identified in the genome-based evaluation. Their membrane sugar transporter and phosphotransferase enzyme coding genes may place them among the hydrolyzing bacteria. *Fermentimonas* and *Lentimicrobium* genera are also among the abundant hydrolyzer core microbes (Figure 5). *Fermentimonas caenicola* (Bin 21) and *Lentimicrobium saccharophilum* (Bin 8) assembled



**FIGURE 4 |** Anvi'o interactive display of automated binning based manual assembly results. The dendrogram in the center display the hierarchical clustering of contigs based on its sequence compositions. The layers from inside out are: (1) The parent layer that marks the splits originated from the same contigs with gray bars (split size 20,000 bps, e.g., Bin 2). (2) The Kaiju taxonomic layer shows the taxonomy for each open reading frame detected in a given split. (3) The length layer, shows the actual length of a given split. (4) GC-content layer. (5) Ribosomal RNA layer. (6) Gray layers represent a sample and each black bar is the coverage of a given split in a given sample. Orange bars represent SNVs detected, and blue bars shows the number of reads mapped in samples. CONCOCT, MAXBIN 2, METABAT 2 shows the automated binning and MANUAL layer the automated based manual binning results. Each bins and the corresponding taxa are listed around the figure.

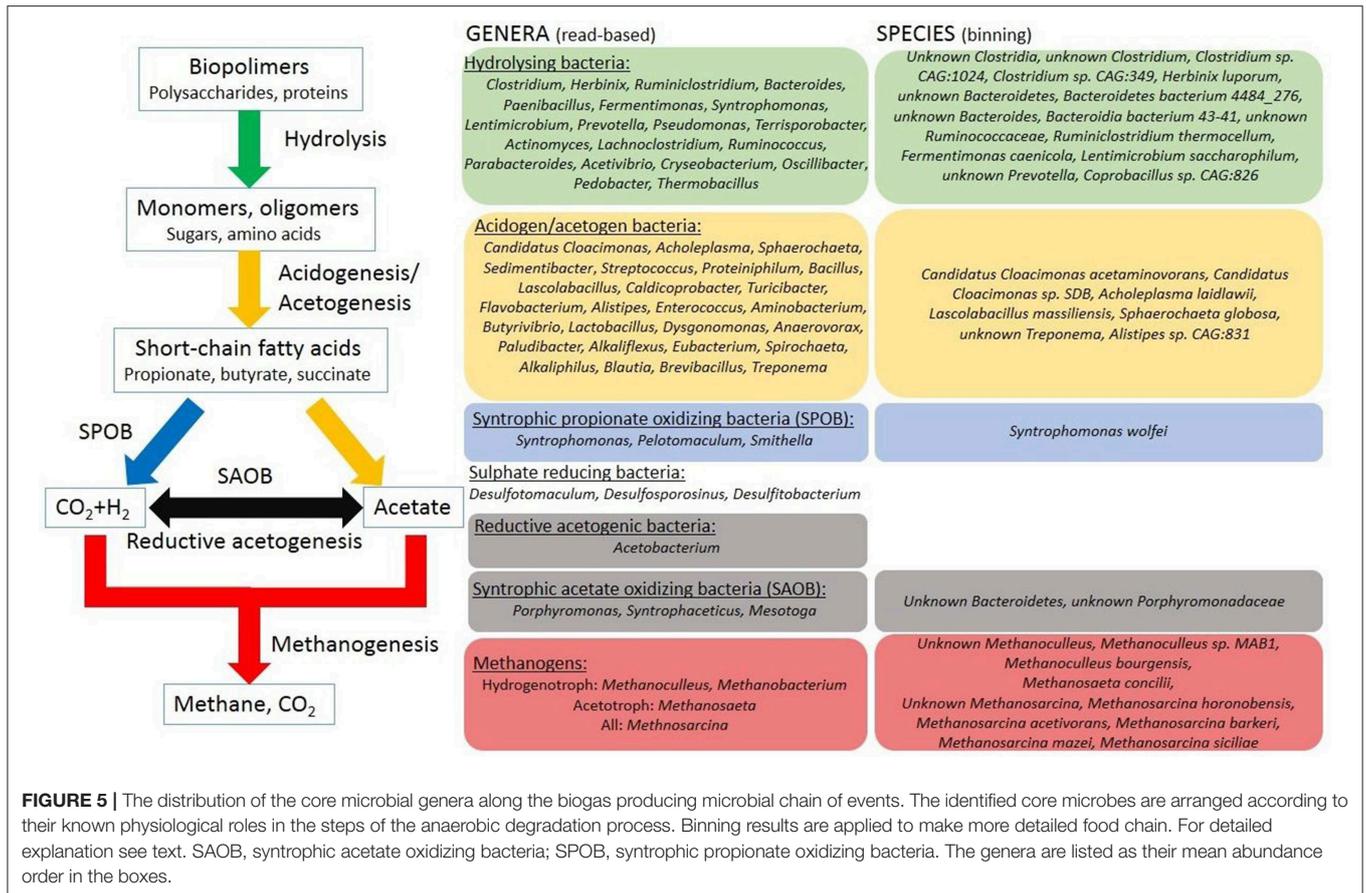
genome fragments fit into this picture (Figure 4 and Supplementary Table 4).

The genus *Prevotella* is a highly diverse taxon comprising various functional niches in different systems through their remarkable metabolic versatility (Matsui et al., 2000; Purushe et al., 2010). *Unknown Prevotella* (Bin 45) and the two *Peptococcaceae bacterium 1109* (Bin 41, 42) and the *Coprobacillus* sp. CAG:826 (Bin 58) represents this group in the genome-based evaluation (Figures 4, 5).

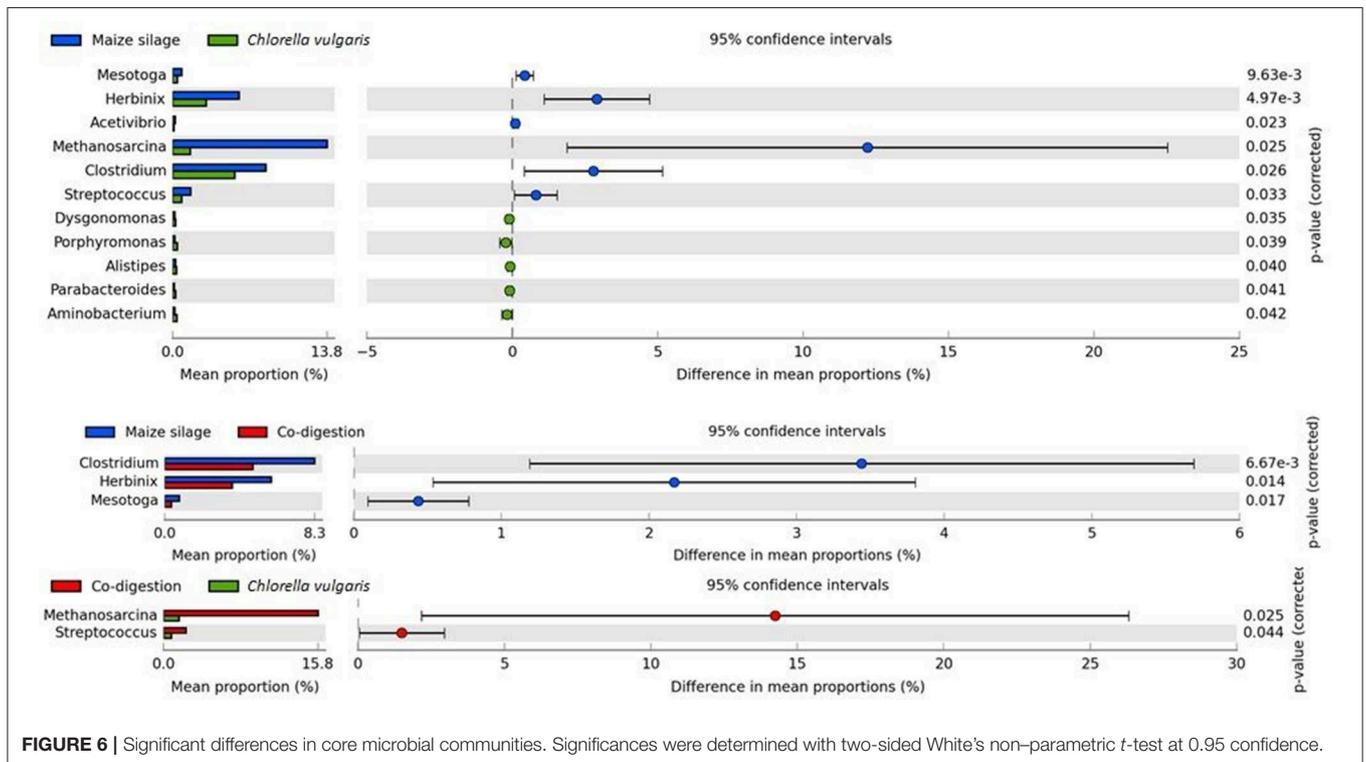
Genera *Paenibacillus*, *Pseudomonas*, *Terrisporobacter*, *Actinomyces*, *Geofilum*, *Lachnoclostridium*, *Cryseobacterium*, *Oscillibacter*, *Acetivibrio*, and *Parabacteroides* were found in lower abundances among core microbes (Nelson et al., 2011; Shida et al., 2012; Lee et al., 2013; Yan et al., 2013; Cho et al.,

2015; Pap et al., 2015; Sun et al., 2016; Gagliano et al., 2017; Lemos et al., 2017; Nakajima et al., 2017; Neshat et al., 2017; Tian et al., 2017) (Supplementary Table 2).

The core genus, *Candidatus Cloacamonas* belongs in the WWE1 candidate division (Pelletier et al., 2008) and has been found primarily in AD reactors operating with pig slurry (Kröber et al., 2009; Wirth et al., 2012). This genus predominated by a large margin in our three digesters (Supplementary Table 2, Figure 3). Automated binning and subsequent manual assembly resulted in 2 medium quality (Bins 11 and 65) and one low quality genome fragment (Bin 12) associated with this group (Bowers et al., 2017) (Figure 4). Tetranucleotide frequency and single copy gene content positively placed the two bins of *CC. acidaminovorans* separately on the clustering



**FIGURE 5 |** The distribution of the core microbial genera along the biogas producing microbial chain of events. The identified core microbes are arranged according to their known physiological roles in the steps of the anaerobic degradation process. Binning results are applied to make more detailed food chain. For detailed explanation see text. SAOB, syntrophic acetate oxidizing bacteria; SPOB, syntrophic propionate oxidizing bacteria. The genera are listed as their mean abundance order in the boxes.



**FIGURE 6 |** Significant differences in core microbial communities. Significances were determined with two-sided White's non-parametric *t*-test at 0.95 confidence.

dendrogram, confirmed by all three binning software (**Figure 4**). Moreover, average nucleotide identity calculation showed that Bin 11 and Bin 65 were only 68% similar to each other (**Supplementary Table 4**) (Yoon et al., 2017). Therefore, these two bins identified two separate *CC. acidaminovorans*-like species. The presence of *Candidatus Cloacimonas* sp. *SDB* (Bin 12) confirmed this observation. Bins 11 and 65 both contain genes coding for enzymes participating in the Embden-Meyerhof pathway. In addition, they may produce proteases for biomass degradation and can be involved in a syntrophic metabolism with methanogens (**Supplementary Table 4**) (Pelletier et al., 2008; Kröber et al., 2009). In addition to *Candidatus Cloacimonas*, a broad diversity of genera took part in acidogenesis/acetogenesis (yellow box in **Figure 5**) (Hofstad et al., 2000; Bosshard et al., 2002; Sundh et al., 2003; Zhilina et al., 2004; Makarova et al., 2006; Ueki et al., 2006; Wade, 2006; Liu et al., 2008; Guerra et al., 2010; Cibis et al., 2016; Stolze et al., 2016; Li et al., 2017; Zhang et al., 2017). Their abundance did not change substantially in the reactors supplied with the assorted feedstocks.

The core oligosaccharide utilizer and acid producer group was represented by *Acholeplasma laidlawii* (Bin 26), *Lascolabacillus massiliensis* (Bin 22), *Sphaerochaeta globosa* (Bin 37), *Unknown Treponema* (Bin 52), and *Treponema brennaborensis* (Bin 51). Read-based metagenomics data showed that the genus *Streptococcus* had low abundance in the digesters fed with microalgae as a monosubstrate (**Figures 5, 6**). *Unknown Streptococcus* (Bin 34) was identified as corresponding genome fragment in genome-based analysis (**Figure 4** and **Supplementary Table 4**). The minor saccharolytic genera *Dysgonomonas* and *Alistipes* were more abundant in the *C. vulgaris* fed reactor. From the members of the genus *Alistipes* (read-based), *Alistipes* sp. *CAG:831* (Bin 53) was detected by the binning approach. Bins 48, 50, and 59 could be identified as *unknown Candidatus zambryskibacteria*, *Acidaminococcus* sp. *CAG:917*, and *Anaerolinea bacterium 49\_20* were additional oligosaccharide degraders. *Unknown Porphyromonadaceae* (Bin 20), *unknown Clostridia* (Bin 18), *Peptococcaceae bacterium* sp. *1109* (Bin 41) contained formyltetrahydrofolate synthetase (FTHFS), the indicator enzyme of anaerobic acetate oxidation, therefore these are possible Syntrophic Acetate Oxidizing Bacteria (SAOB) (gray box in **Figure 5**) (see section Metagenome Based Functional Changes). The genera *Syntrophaceticus* and *Mesotoga* are likely to have SAOB function, although bins representing them were not found, therefore this finding is not supported by the genome-based evaluation of data (Westerholm et al., 2010; Wang et al., 2013; Nobu et al., 2015; Müller et al., 2016). Syntrophic Propionate Oxidizing Bacteria (SPOB) were observed in all three reactors at all sampling time points by read-based analysis (*Syntrophomonas*, *Pelotomaculum*, and *Smithella*). These genera are able to convert propionate to acetate, H<sub>2</sub> and CO<sub>2</sub> (Lueders et al., 2004; Kosaka et al., 2008) in syntrophy with hydrogenotrophic methanogens (blue box in **Figure 5**). Indeed, *Syntrophomonas wolfei* (Bin 33) came up in the genome-based evaluation as a well-known SPOB bacterium (**Figure 4**) (McInerney et al., 1981; Popp et al., 2017). The low abundance of the genera *Pelotomaculum* and *Smithella* may explain why these taxa are missing from the binning results.

Similarly, low abundance characterized the sulfate reducing bacteria *Desulfotomaculum*, *Desulfosporosinus*, and *Desulfitobacterium* (Friedrich et al., 1996; Pester et al., 2012) in the read-based metagenomes. These microbes may represent an additional H<sub>2</sub> sink in AD. They were also present in all reactors examined and were part of the core microbiome as permanent rivals of reductive homoacetogens and hydrogenotrophic methanogens for H<sub>2</sub> (Liu and Lu, 2018) (**Supplementary Table 2**). Nevertheless, sulfate reducers were not found in the genome-based evaluation.

Methanogenic Archaea populate the last box in biogas formation, highlighted with red in **Figure 5**. In the inoculum, the genus *Methanosaeta* was the predominating archaeon over *Methanoculleus*, *Methanosarcina*, and *Methanobacterium* (**Supplementary Figure 2**). The genera *Methanoculleus* and *Methanobacterium* are hydrogenotrophic, while the genus *Methanosaeta* comprise obligate acetoclastic methanogens. Among hydrogenotrophic archaea the genus *Methanoculleus* was represented by *Methanoculleus bourgensis* (Bin 38), *unknown Methanoculleus* (Bin 39) and *Methanoculleus* sp. *MAB1* (Bin 40) and the genus *Methanobacterium* by *unknown Methanobacterium* (Bin 32). A member of the genus *Methanosaeta* was identified as *Methanosaeta concilii* (Bin 47) among acetoclastic methanogens (**Figure 4**). *Methanosarcina* is the only known genus able to produce methane through all three methanogenic pathways, i.e., acetoclastic, hydrogenotroph, and methylotroph (Smith and Ingram-Smith, 2007; Hook et al., 2010; Nettmann et al., 2010; Maus et al., 2012). Several species were detected from the genus *Methanosarcina* in the binning study. The presence of *Unknown Methanosarcina* (Bin 64), *Methanosarcina horonobensis* (Bin 63), *Methanosarcina mazei* (Bin 62), *Methanosarcina acetivorans* (Bin 60), *Methanosarcina siciliae* (Bin 61) indicated that this group was probably the most diverse among methanogens in our system (**Figure 5**). *Methanosaeta* remained predominant in the *C. vulgaris* fed digester, but their relative number of methanogens decreased in time (**Supplementary Figure 2**). The genus *Methanosarcina* replaced *Methanosaeta* in abundance in the case of maize silage and co-digestion as the experiment progressed (Ellis et al., 2012; Wirth et al., 2012, 2015a; Pope et al., 2013; Klassen et al., 2016) (**Figure 6** and **Supplementary Figure 2**). In earlier studies, both *Methanosarcina* and *Methanosaeta* have been reported to be dominant methanogens (Zamalloa et al., 2012; Gonzalez-Fernandez et al., 2015; Klassen et al., 2016), depending primarily on the actual acetate concentration. *Methanosaeta* outcompeted *Methanosarcina* at low acetate concentration, while *Methanosarcina* predominated *Methanosaeta* at high acetate levels (Smith and Ingram-Smith, 2007; De Vrieze et al., 2012). Other operational parameters, e.g., NH<sub>3</sub> concentration, may also change the equilibrium between these two genera (Chen and He, 2015; Leite et al., 2015; Narihiro et al., 2015; Chen et al., 2017). The genus *Methanosarcina* is more tolerant to ammonia, than *Methanosaeta* (Fotidis et al., 2013; Gao et al., 2015; Niu et al., 2015). This may explain the decrease of relative abundance of methanogens during *C. vulgaris* AD. Adaptation of methanogens to high ammonia concentration could be one solution of ammonia inhibition (Kovács E.

et al., 2013; Rajagopal et al., 2013; Mahdy et al., 2017) (**Supplementary Figure 3**).

It is noteworthy that the following precautions have to be kept in mind when the above characterization of the core microbiome is interpreted. (1) The 92-member core genera seem sufficient to cover all basic metabolic steps necessary for biogas formation from the green alga and maize silage. This does not mean, however, that this diversity is the minimum requirement for successful degradation of organic matter and concomitant biogas production (Calusinska et al., 2018; Campanaro et al., 2018a). (2) Here we could reach the genus level resolution of the microbiota by the read-based metagenomes. Some genera have fairly diverse species composition leading to diverse properties of the taxon. The analysis of the community by genome-based evaluation may help to make more precise understanding of the abundant taxa. Nevertheless, depending on the sequence number and the observed microbiota complexity only the relatively abundant sequences can be compiled in MAGs (metagenome-assembled genomes) or quality genome fragments (Bowers et al., 2017). (3) The information content of the available sequence databases is limited, a portion of the sequenced but not classified reads may belong to “microbial dark matter,” e.g., “unknown” bins such as Bin 5, 15, 17, 29, 31, 36, 46, 57 (Hatfull, 2015; Nobu et al., 2015; Solden et al., 2016).

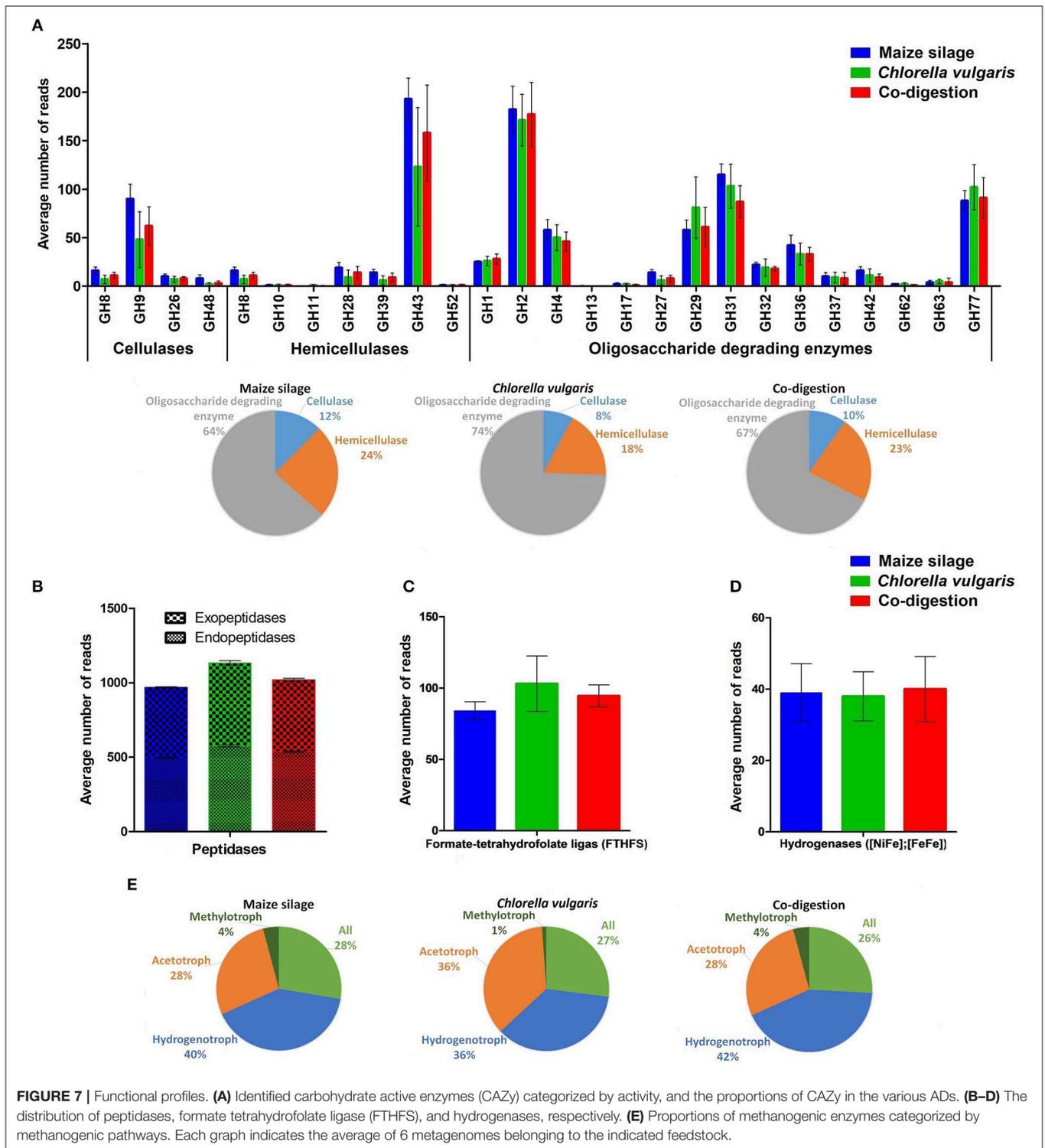
## Metagenome Based Functional Changes

The metagenomics data were also used to extract potential functional information about the biological activity of the AD communities fed with the various feedstock combinations. Genes coding for characteristic and signature proteins have been detected and their relative copy number in the samples was determined by counting the reads related to the gene in question. This information was corroborated by the binning study, which helped to associate specific functions to taxa. Although the presence of a certain gene and the number of reads linked to it does not necessarily mean that the gene is being transcribed and translated, it is safe to assume at least a limited correlation between these parameters (Wirth et al., 2018). The main functional profiles investigated were the carbohydrate-active enzymes (CAZy), protein degradation, syntrophic acetate oxidation, i.e., formate-tetrahydrofolate ligase, hydrogen metabolism and methanogenesis (**Figure 7**) (Hattori, 2008; Caspi et al., 2014; Lombard et al., 2014; Rawlings et al., 2018). The glycoside hydrolase families (GH) catalyze the breakdown of poly- and oligosaccharides. GH9 predominated the cellulase group. This enzyme family was found in our *unknown Bacteroidetes* (Bin 13), *unknown Porphyromonadaceae* (Bin 20), *Herbinix luporum* (Bin 25), *unknown Bacteroides* (Bin 43), and in *unknown Bacteroidia* (Bin 44) assembled genome fragments (**Supplementary Table 4**). GH9 has been identified in numerous biogas producing ecosystems (Wei et al., 2015; Güllert et al., 2016; Maus et al., 2016). The GH8, GH9, and GH39 families were detected among the core functions (**Supplementary Table 3**, green color). As a tendency, the GH genes coding for cellulases were more abundant in the reactors running on maize silage or co-digestion than in the *C. vulgaris* fed one.

The GH43 enzyme family emerged from the hemicellulases and was found in the *unknown Porphyromonadaceae* (Bin 20), *Fermentimonas caenicola* (Bin 21), *Lascolabacillus massiliensis* (Bin 22), *Herbinix luporum* (Bin 25), *Peptococcaceae bacterium 1109* (Bins 41 and 42), *unknown Bacteroides* (Bin 43), and *Unknown Prevotella* (Bin 45). The GH43 family was identified as one of the 15 most abundant GH families in a thermophilic biogas plant (Maus et al., 2016). The richness of cellulases and hemicellulases were reduced in the reactors fed with *C. vulgaris* biomass. The large number of oligosaccharide-degrading GH enzymes covers a wide range of catalytic activity (**Figure 7A**). GH2, GH4, GH29, GH31, GH42, and GH77 were present in all sequenced samples although none of them showed significant ( $p < 0.02$ ) differences among the various reactor functional profiles. The GH2 and GH31 families were detected in the bins of the *unknown Bacteroidetes* (Bin 6 and 13), *Bacteroidetes bacterium 4484\_276* (Bin 7), *unknown Porphyromonadaceae* (Bin 20), *Fermentimonas caenicola* (Bin 21), *Peptococcaceae bacterium 1109* (Bin 41), *unknown Bacteroides* (Bin 43), *unknown Prevotella* (Bin 45) and in two unknown assemblies (Bin 17 and 31) (**Supplementary Table 4**). It should be noted that not all the GH families presented in **Figure 7A** are part of the core functions, which are compiled in **Supplementary Table 3**, highlighted in green color. In general, maize silage and co-digestion appeared to develop similar CAZY profiles.

Based on InterPro data and the EMBL-EBI MERPOS peptidase database classification, a large number of endo- and exopeptidases were present in our digesters (**Supplementary Table 3**, highlighted in yellow color) (Rawlings et al., 2018). The great variety of peptidases identified by read-based metagenomics was confirmed by binning results. The genes involved in protein degradation were higher in representation in the reactors digesting *C. vulgaris* than those fed with maize silage (**Figure 7B**). The known functions of the peptidases in family S51 are nutritional; lack of these enzymes leads to incomplete degradation of intracellular proteins to amino acids (Lassy and Miller, 2000). This peptidase was found in the *unknown Bacteroidetes* (Bin 6) and *unknown Porphyromonadaceae* (Bin 20) assembled genomes. The peptidase family M20 was present in the largest abundance among functions (**Supplementary Table 3**, highlighted in yellow color) in our data set. It was detected in the following 14 bins: *unknown Firmicutes* (Bin 4), *unknown Bacteroidetes* (Bin 6), *Sedimentibacter* sp. B4 (Bin 16), *unknown Porphyromonadaceae* (Bin 20), *Lascolabacillus massiliensis* (Bin 22), *unknown Clostridia* (Bin 30), unknown (Bin 31), *Peptococcaceae bacterium 1109* (Bin 42), *unknown Bacteroides* (Bin 43), *Methanosaeta concilii* (Bin 47), *Coprocbacillus* sp. CAG:826 (Bin 58), *Methanosarcina horonobensis* (Bin 63), *unknown Methanosarcina* (Bin 64) and one of the *Candidatus Cloacimonas acetaminovorans* bins (Bin 65). In general, peptidases in the M20 family are hydrolases to complete the conversion of proteins to free amino acids (Martínez-Rodríguez et al., 2012).

Formate-tetrahydrofolate ligase (i.e., formyltetrahydrofolate synthetase) (FTHFS) was also among the selected signature enzymes. FTHFS is a highly expressed key enzyme in both the Wood-Ljungdahl pathway of autotrophic CO<sub>2</sub> fixation



**FIGURE 7 |** Functional profiles. **(A)** Identified carbohydrate active enzymes (CAZy) categorized by activity, and the proportions of CAZy in the various ADs. **(B–D)** The distribution of peptidases, formate tetrahydrofolate ligase (FTHFS), and hydrogenases, respectively. **(E)** Proportions of methanogenic enzymes categorized by methanogenic pathways. Each graph indicates the average of 6 metagenomes belonging to the indicated feedstock.

(acetogenesis) and the glycine synthase/reductase pathway of purinolysis. The key physiological role of this enzyme in acetogens is to catalyze the formylation of tetrahydrofolate, an initial step in the reduction of CO<sub>2</sub> and other one-carbon precursors to acetate. In purinolytic organisms, the

enzymatic reaction is reversed, i.e., it releases formate from 10-formyltetrahydrofolate with concurrent production of ATP (<http://pfam.xfam.org/family/PF01268>). The detailed biochemical and regulatory mechanisms behind the shift and the way in which the bacteria regain energy is uncertain in spite

of intensive investigations (Pester and Brune, 2006; Ragsdale and Pierce, 2009; Xu et al., 2009). FTHFS was present among the putative core functions (**Supplementary Table 3**, gray color) and its relative abundance was somewhat elevated in the reactors digesting *C. vulgaris* relative to the ones fed with maize silage and/or co-digestion (**Figure 7C**). The FTHFS gene was present in *unknown Clostridia* (Bin 18), *unknown Porphyromonadaceae* (Bin 20), *Peptococcaceae bacterium 1109* (Bin 41), and in *Candidatus Cloacimonas acetaminovorans* (Bin 65) assemblies.

The equilibrium between H<sub>2</sub> producers and consumers is crucial in methanogenesis (Wirth et al., 2012; Szuhaj et al., 2016; Bagi et al., 2017). The relative numbers of identified hydrogenase genes were nearly equal in the three reactors (**Figure 7D**) (**Supplementary Table 3**, purple color). The gene coding for [FeFe] hydrogenase maturation, HydE was among the predicted core functions. The HydE protein, together with HydF and HydG comprise the accessory gene products for the assembly of an active [FeFe] hydrogenase (King et al., 2006). Genes coding for enzymes involved in iron hydrogenase maturation were found in the metagenome assembled genomes of *unknown Bacteroidetes* (Bin 6 and 13), *Herbinix luporum* (Bin 25), *Ruminiclostridium thermocellum* (Bin 27), and in both *Candidatus Cloacimonas acetaminovorans* (Bins 11 and 65). The observation suggests an equally important role of H<sub>2</sub> metabolism in biogas formation, independent of the feedstock composition.

The genes coding for enzymes involved in the biogas evolution pathways were our final group of signature functions tested. A collection of 24 methanogenic enzymes were identified (**Supplementary Table 3**, red color); 14 of them were present in the core. Hydrogenotrophic methanogenesis predominated in maize silage and co-digestion. In contrast, acetoclastic methanogenesis was present in higher proportion when only *C. vulgaris* was the feedstock (**Figure 7E**). Among the core methanogenic enzymes, methyl coenzyme M reductase (subunit A, B, G, and D) and CoB–CoM heterodisulfide reductase (subunit B) were detected. Methyl coenzyme M reductase genes were observed in *unknown Methanoculleus* (Bin 39), *Methanosarcina concilii* (Bin 47), *unknown Methanosarcina* (Bin 64) (**Supplementary Table 4**). The methyl coenzyme M reductase catalyzes the reduction of methyl-coenzyme M and coenzyme B to methane (Luton et al., 2002; Nunoura et al., 2008).

## REFERENCES

- Afgan, E., Baker, D., van den Beek, M., Blankenberg, D., and Bouvier, D., Cech, M., et al. (2016). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic Acids Res.* 44, W3–W10. doi: 10.1093/nar/gkw343
- Agarwala, R., Barrett, T., Beck, J., Benson, D. A., Bollin, C., Bolton, E., et al. (2018). Database resources of the national center for biotechnology information. *Nucleic Acids Res.* 46, D8–D13. doi: 10.1093/nar/gkv1290
- Alneberg, J., Bjarnason, B. S., de Bruijn, I., Schirmer, M., Quick, J., Ijaz, U. Z., et al. (2013). CONCOCT: clustering cONTigs on COverage and ComposiTiOn. *arXiv* 1–28.
- Alzate, M. E., Muñoz, R., Rogalla, F., Fdz-Polanco, F., and Pérez-Elvira, S. I. (2012). Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. *Bioresour. Technol.* 123, 488–494. doi: 10.1016/j.biortech.2012.06.113
- Amon, T., Amon, B., Kryvoruchko, V., Zollitsch, W., Mayer, K., and Gruber, L. (2007). Biogas production from maize and dairy cattle manure-Influence of biomass composition on the methane yield. *Agric. Ecosyst. Environ.* 118, 173–182. doi: 10.1016/j.agee.2006.05.007
- Bagi, Z., Ács, N., Böjti, T., Kakuk, B., and Rákhely, G., Strang, O., et al. (2017). Biomethane: the energy storage, platform chemical and greenhouse gas mitigation target. *Anaerobe* 46, 13–22. doi: 10.1016/j.anaerobe.2017.03.001
- Becker, S., Böger, P., Oehlmann, R., and Bo, P. (2000). PCR Bias in ecological analysis : a case study for quantitative Taq nuclease assays in analyses of microbial communities. *Appl. Environ. Microbiol.* 66, 4945–4953. doi: 10.1128/AEM.66.11.4945-4953.2000

## DATA AVAILABILITY STATEMENT

Sequences are available on MG-RAST under the study name: “Anaerobic digestion of *C. vulgaris* microalgal biomass” (ID: mgp20184). The data are also available in NCBI Sequence Read Archive (SRA) under the submission number: PRJNA544905.

## AUTHOR CONTRIBUTIONS

RW with the help from TB and GL, developed the DNA extraction protocol, designed and performed the experiments, and contributed to the evaluation of metagenomic data. GM organized and performed the DNA sequencing work. KK and ZB conceived the project and participated in its design. RW and KK drafted the manuscript. GR critically evaluated the manuscript. All the authors have read and approved the final manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenrg.2019.00111/full#supplementary-material>

- Bosshard, P. P., Zbinden, R., and Altwegg, M. (2002). *Turicibacter sanguinis* gen. nov., sp. nov., a novel anaerobic, Gram-positive bacterium. *Int. J. Syst. Evol. Microbiol.* 12, 1263–1266. doi: 10.1099/00207713-52-4-1263
- Bowers, R. M., Kyrpides, N. C., Stepanauskas, R., Harmon-Smith, M., Doud, D., Reddy, T. B. K., et al. (2017). Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. *Nat. Biotechnol.* 35, 725–731. doi: 10.1038/nbt.3893
- Bray, J. R., and Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecol. Monogr.* 27, 325–349. doi: 10.2307/1942268
- Buchfink, B., Xie, C., and Huson, D. H. (2014). Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* 12, 59–60. doi: 10.1038/nmeth.3176
- Cai, M., Wilkins, D., Chen, J., Ng, S. K., Lu, H., Jia, Y., et al. (2016). Metagenomic reconstruction of key anaerobic digestion pathways in municipal sludge and industrial wastewater biogas-producing systems. *Front. Microbiol.* 7:778. doi: 10.3389/fmicb.2016.00778
- Calusinska, M., Goux, X., Fossépré, M., Muller, E. E. L., Wilmes, P., and Delfosse, P. (2018). A year of monitoring 20 mesophilic full-scale bioreactors reveals the existence of stable but different core microbiomes in bio-waste and wastewater anaerobic digestion systems. *Biotechnol. Biofuels* 11:196. doi: 10.1186/s13068-018-1195-8
- Campanaro, S., Treu, L., Kougias, P. G., De Francisci, D., Valle, G., and Angelidaki, I. (2016). Metagenomic analysis and functional characterization of the biogas microbiome using high throughput shotgun sequencing and a novel binning strategy. *Biotechnol. Biofuels* 9:26. doi: 10.1186/s13068-016-0441-1
- Campanaro, S., Treu, L., Kougias, P. G., Luo, G., and Angelidaki, I. (2018a). Metagenomic binning reveals the functional roles of core abundant microorganisms in twelve full-scale biogas plants. *Water Res.* 140, 123–134. doi: 10.1016/j.watres.2018.04.043
- Campanaro, S., Treu, L., Kougias, P. G., Zhu, X., and Angelidaki, I. (2018b). Taxonomy of anaerobic digestion microbiome reveals biases associated with the applied high throughput sequencing strategies. *Sci. Rep.* 8:1926. doi: 10.1038/s41598-018-20414-0
- Campbell, J. H., O'Donoghue, P., Campbell, A. G., Schwientek, P., Sczyrba, A., Woyke, T., et al. (2013). UGA is an additional glycine codon in uncultured SR1 bacteria from the human microbiota. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5540–5545. doi: 10.1073/pnas.1303090110
- Carrere, H., Antonopoulou, G., Affes, R., Passos, F., Battimelli, A., Lyberatos, G., et al. (2016). Review of feedstock pretreatment strategies for improved anaerobic digestion: from lab-scale research to full-scale application. *Bioresour. Technol.* 199, 386–397. doi: 10.1016/j.biortech.2015.09.007
- Caspi, R., Altman, T., Dale, J. M., Dreher, K., Fulcher, C. A., Gilham, F., et al. (2014). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases. *Nucleic Acids Res.* 38, 459–471. doi: 10.1093/nar/gkt1103
- Chen, S., Cheng, H., Liu, J., Hazen, T. C., Huang, V., and He, Q. (2017). Unexpected competitiveness of *Methanoseta* populations at elevated acetate concentrations in methanogenic treatment of animal wastewater. *Appl. Microbiol. Biotechnol.* 101, 1729–1738. doi: 10.1007/s00253-016-7967-9
- Chen, S., and He, Q. (2015). Persistence of *Methanoseta* populations in anaerobic digestion during process instability. *J. Ind. Microbiol. Biotechnol.* 42, 1129–1137. doi: 10.1007/s10295-015-1632-7
- Chen, X., Li, Z., He, N., Zheng, Y., Li, H., Wang, H., et al. (2018). Nitrogen and phosphorus removal from anaerobically digested wastewater by microalgae cultured in a novel membrane photobioreactor. *Biotechnol. Biofuels* 11:190. doi: 10.1186/s13068-018-1190-0
- Chen, Y., Cheng, J. J., and Creamer, K. S. (2008). Inhibition of anaerobic digestion process: a review. *Bioresour. Technol.* 99, 4044–4064. doi: 10.1016/j.biortech.2007.01.057
- Cho, H. U., Kim, Y. M., Choi, Y.-N., Kim, H. G., and Park, J. M. (2015). Influence of temperature on volatile fatty acid production and microbial community structure during anaerobic fermentation of microalgae. *Bioresour. Technol.* 191, 475–480. doi: 10.1016/j.biortech.2015.03.009
- Cibis, K. G., Gneipel, A., and König, H. (2016). Isolation of acetic, propionic and butyric acid-forming bacteria from biogas plants. *J. Biotechnol.* 220, 51–63. doi: 10.1016/j.jbiotec.2016.01.008
- Córdova, O., Chamy, R., Guerrero, L., and Sánchez-Rodríguez, A. (2018). Assessing the effect of pretreatments on the structure and functionality of microbial communities for the bioconversion of microalgae to biogas. *Front. Microbiol.* 9:1388. doi: 10.3389/fmicb.2018.01388
- De Vrieze, J., Gildemyn, S., Vilchez-Vargas, R., Jáuregui, R., Pieper, D. H., Verstraete, W., et al. (2014). Inoculum selection is crucial to ensure operational stability in anaerobic digestion. *Appl. Microbiol. Biotechnol.* 99, 189–199. doi: 10.1007/s00253-014-6046-3
- De Vrieze, J., Hennebel, T., Boon, N., and Verstraete, W. (2012). Methanosarcina: the rediscovered methanogen for heavy duty biometathation. *Bioresour. Technol.* 112, 1–9. doi: 10.1016/j.biortech.2012.02.079
- De Vrieze, J., Regueiro, L., Props, R., Vilchez-Vargas, R., Jáuregui, R., Pieper, D. H., et al. (2016). Presence does not imply activity: DNA and RNA patterns differ in response to salt perturbation in anaerobic digestion. *Biotechnol. Biofuels* 9:244. doi: 10.1186/s13068-016-0652-5
- Debowski, M., Zielinski, M., Grala, A., and Dudek, M. (2013). Algae biomass as an alternative substrate in biogas production technologies - review. *Renew. Sustain. Energy Rev.* 27, 596–604. doi: 10.1016/j.rser.2013.07.029
- Delmont, T. O., Quince, C., Shaiber, A., Esen, Ö. C., Lee, S. T., Rappé, M. S., et al. (2018). Nitrogen-fixing populations of Planctomycetes and Proteobacteria are abundant in surface ocean metagenomes. *Nat. Microbiol.* 3, 804–813. doi: 10.1038/s41564-018-0176-9
- Demuez, M., Mahdy, A., Tomás-Pejoj, E., González-Fernández, C., and Ballesteros, M. (2015). Enzymatic cell disruption of microalgae biomass in biorefinery processes. *Biotechnol. Bioeng.* 112, 1955–1966. doi: 10.1002/bit.25644
- Ellis, J. T., Tramp, C., Sims, R. C., Miller, C. D., et al. (2012). Characterization of a methanogenic community within an algal fed anaerobic digester. *ISRN Microbiol.* 2012, 1–12. doi: 10.5402/2012/517524
- Eren, A. M., Esen, Ö. C., Quince, C., Vineis, J. H., Morrison, H. G., Sogin, M. L., et al. (2015). Anvi'o: an advanced analysis and visualization platform for 'omics data. *PeerJ* 3:e1319. doi: 10.7717/peerj.1319
- Estime, B., Ren, D., and Sureshkumar, R. (2017). Cultivation and energy efficient harvesting of microalgae using thermoreversible sol-gel transition. *Sci. Rep.* 7:40725. doi: 10.1038/srep40725
- Federhen, S. (2015). The NCBI taxonomy database. *Nucleic Acids Res.* 43, D1086–D1098. doi: 10.1093/nar/gku1127
- Finn, D. R., Clements, J., and Eddy, R. S. (2011). HMMER web server: interactive sequence similarity searching. *Nucleic Acids Res.* 39, W29–W37. doi: 10.1093/nar/gkr367
- Finn, R. D., Attwood, T. K., Babbitt, P. C., Bateman, A., Bork, P., Bridge, A. J., et al. (2017). InterPro in 2017-beyond protein family and domain annotations. *Nucleic Acids Res.* 45, D190–D199. doi: 10.1093/nar/gkw1107
- Finn, R. D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R. Y., Eddy, S. R., et al. (2014). Pfam: the protein families database. *Nucleic Acids Res.* 42, 222–230. doi: 10.1093/nar/gkt1223
- Fotidis, I. A., Karakashev, D., Kotsopoulos, T. A., Martzopoulos, G. G., and Angelidaki, I. (2013). Effect of ammonium and acetate on methanogenic pathway and methanogenic community composition. *FEMS Microbiol. Ecol.* 83, 38–48. doi: 10.1111/j.1574-6941.2012.01456.x
- Friedrich, M., Springer, N., Ludwig, W., and Schink, B. (1996). Phylogenetic positions of *Desulfofustis glycolicus* gen. nov., sp. nov., and nov *Syntrophobotulus glycolicus* gen. nov., sp., two new strict anaerobes growing with glycolic acid. *Int. J. Syst. Bacteriol.* 46, 1065–1069. doi: 10.1099/00207713-46-4-1065
- Gagliano, M. C., Ismail, S. B., Stams, A. J. M., Plugge, C. M., Temmink, H., and Van Lier, J. B. (2017). Biofilm formation and granule properties in anaerobic digestion at high salinity. *Water Res.* 121, 61–71. doi: 10.1016/j.watres.2017.05.016
- Gao, S., Zhao, M., Chen, Y., Yu, M., and Ruan, W. (2015). Tolerance response to *in situ* ammonia stress in a pilot-scale anaerobic digestion reactor for alleviating ammonia inhibition. *Bioresour. Technol.* 198, 372–379. doi: 10.1016/j.biortech.2015.09.044
- Goecks, J., Nekrutenko, A., Taylor, J., Afgan, E., Ananda, G., Baker, D., et al. (2010). Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol.* 11:R86. doi: 10.1186/gb-2010-11-8-r86
- Gonzales-Fernandez, C., Barreiro-Vescovo, S., de Godos, I., Fernandez, M., Zouhayr, A., and Ballesteros, M. (2018). Biochemical methane potential of microalgae biomass using different microbial inocula. *Biotechnol. Biofuels* 11:184. doi: 10.1186/s13068-018-1188-7

- Gonzalez-Fernandez, C., Sialve, B., and Molinuevo-Salces, B. (2015). Anaerobic digestion of microalgal biomass: challenges, opportunities and research needs. *Bioresour. Technol.* 198, 896–906. doi: 10.1016/j.biortech.2015.09.095
- Guerra, N. P., Fajardo, P., Fuciños, C., Amado, I. R., Alonso, E., Torrado, A., et al. (2010). Modelling the biphasic growth and product formation by *Enterococcus faecium* CECT 410 in realcalized fed-batch fermentations in whey. *J. Biomed. Biotechnol.* 2010:290286. doi: 10.1155/2010/290286
- Guldhe, A., Kumari, S., Ramana, L., Ramsundar, P., Singh, P., Rawat, I., et al. (2017). Prospects, recent advancements and challenges of different wastewater streams for microalgal cultivation. *J. Environ. Manage.* 203, 299–315. doi: 10.1016/j.jenvman.2017.08.012
- Güllert, S., Fischer, M. A., Turaev, D., Noebauer, B., Ilmberger, N., Wemheuer, B., et al. (2016). Deep metagenome and metatranscriptome analyses of microbial communities affiliated with an industrial biogas fermenter, a cow rumen, and elephant feces reveal major differences in carbohydrate hydrolysis strategies. *Biotechnol. Biofuels* 9, 1–20. doi: 10.1186/s13068-016-0534-x
- Gunaseelan, V. N. (1997). Anaerobic digestion of biomass for methane production: a review. *Biomass Bioener.* 13, 83–114. doi: 10.1016/S0961-9534(97)00020-2
- Han, S., Liu, Y., Zhang, S., and Luo, G. (2016). Reactor performances and microbial communities of biogas reactors: effects of inoculum sources. *Appl. Microbiol. Biotechnol.* 100, 987–995. doi: 10.1007/s00253-015-7062-7
- Harun, R., Singh, M., Forde, G. M., and Danquah, M. K. (2010). Bioprocess engineering of microalgae to produce a variety of consumer products. *Renew. Sustain. Energy Rev.* 14, 1037–1047. doi: 10.1016/j.rser.2009.11.004
- Hatfull, G. F. (2015). Dark matter of the biosphere: the amazing world of bacteriophage diversity. *J. Virol.* 89, 8107–8110. doi: 10.1128/JVI.01340-15
- Hattori, S. (2008). Syntrophic acetate-oxidizing microbes in methanogenic environments. *Microbes Environ.* 23, 118–127. doi: 10.1264/jsme2.23.118
- Heintz-Buschart, A., May, P., Laczny, C. C., Lebrun, L. A., Bellora, C., Krishna, A., et al. (2016). Integrated multi-omics of the human gut microbiome in a case study of familial type 1 diabetes. *Nat. Microbiol.* 2, 1–12. doi: 10.1038/nmicrobiol.2016.227
- Hijazi, O., Munro, S., Zerhusen, B., and Effenberger, M. (2016). Review of life cycle assessment for biogas production in Europe. *Renew. Sustain. Energy Rev.* 54, 1291–1300. doi: 10.1016/j.rser.2015.10.013
- Hofstad, T., Olsen, I., Eribe, E. R., Falsen, E., Collins, M. D., Lawson, P., et al. (2000). *Dysgonomonas* gen. nov. to accommodate *Dysgonomonas gadei* sp. nov., an organism isolated from a human gall bladder, and *Dysgonomonas capnocytophagoides* (formerly CDC group DF-3). *Int. J. Syst. Evol. Microbiol.* 50, 2189–2195. doi: 10.1099/00207713-50-6-2189
- Holm-Nielsen, J. B., Al Seadi, T., and Oleskowicz-Popiel, P. (2009). The future of anaerobic digestion and biogas utilization. *Bioresour. Technol.* 100, 5478–5484. doi: 10.1016/j.biortech.2008.12.046
- Hook, S. E., Wright, A. D., and McBride, B. W. (2010). Methanogens: methane producers of the rumen and mitigation strategies. *Archaea* 2010, 50–60. doi: 10.1155/2010/945785
- Huse, S. M., Ye, Y., Zhou, Y., and Fodor, A. A. (2012). A core human microbiome as viewed through 16S rRNA sequence clusters. *PLoS ONE* 7:34242. doi: 10.1371/journal.pone.0034242
- Huson, D., Auch, A., Qi, J., and Schuster, S. (2007). MEGAN analysis of metagenome data. *Genome Res.* 17, 377–386. doi: 10.1101/gr.5969107
- Huson, D., Mitra, S., and Ruscheweyh, H. (2011). Integrative analysis of environmental sequences using MEGAN4. *Genome Res.* 21, 1552–1560. doi: 10.1101/gr.120618.111
- Huson, D. H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., et al. (2016). MEGAN community edition - interactive exploration and analysis of large-scale microbiome sequencing data. *PLoS Comput. Biol.* 12:e1004957. doi: 10.1371/journal.pcbi.1004957
- Hyatt, D., Chen, G. L., LoCascio, P. F., Land, M. L., Larimer, F. W., and Hauser, L. J. (2010). Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. doi: 10.1186/1471-2105-11-119
- Jami, E., and Mizrahi, I. (2012). Composition and similarity of bovine rumen microbiota across individual animals. *PLoS ONE* 7:e33306. doi: 10.1371/journal.pone.0033306
- Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., et al. (2014). InterProScan 5: genome-scale protein function classification. *Bioinformatics* 30, 1236–1240. doi: 10.1093/bioinformatics/btu031
- Kang, D. D., Froula, J., Egan, R., and Wang, Z. (2015). MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. *PeerJ* 3:e1165. doi: 10.7717/peerj.1165
- Khalid, A., Arshad, M., Anjum, M., Mahmood, T., and Dawson, L. (2011). The anaerobic digestion of solid organic waste. *Waste Manage.* 31, 1737–1744. doi: 10.1016/j.wasman.2011.03.021
- King, P. W., Posewitz, M. C., Ghirardi, M. L., and Seibert, M. (2006). Functional studies of [FeFe] hydrogenase maturation in an *Escherichia coli* biosynthetic system. *J. Bacteriol.* 188, 2163–2172. doi: 10.1128/JB.188.6.2163-2172.2006
- Klassen, V., Blifernez-Klassen, O., Wobbe, L., Schlüter, A., Kruse, O., and Mussnug, J. H. (2016). Efficiency and biotechnological aspects of biogas production from microalgal substrates. *J. Biotechnol.* 234, 7–26. doi: 10.1016/j.jbiotec.2016.07.015
- Koeck, D. E., Ludwig, W., Wanner, G., Zverlov, V. V., Liebl, W., and Schwarz, W. H. (2015). *Herbinix hemicellulosilytica*, gen. nov., sp. nov., a thermophilic cellulose degrading bacterium isolated from a thermophilic biogas reactor. *Int. J. Syst. Evol. Microbiol.* 65, 2365–2371. doi: 10.1099/ijss.0.000264
- Kosaka, T., Kato, S., Shimoyama, T., Ishii, S., Abe, T., and Watanabe, K. (2008). The genome of *Pelotomaculum thermopropionicum* reveals niche-associated evolution in anaerobic microbiota. *Genome Res.* 18, 442–448. doi: 10.1101/gr.7136508
- Kovács, E., Wirth, R., Maróti, G., Bagi, Z., Rákhely, G., and Kovács, K. L. (2013). Biogas production from protein-rich biomass: fed-batch anaerobic fermentation of casein and of pig blood and associated changes in microbial community composition. *PLoS ONE* 8:e77265. doi: 10.1371/journal.pone.0077265
- Kovács, K. L., Ács, N., Kovács, E., Wirth, R., and Rákhely, G., Strang, O., et al. (2013). Improvement of biogas production by bioaugmentation. *Biomed Res. Int.* 2013:482653. doi: 10.1155/2013/482653
- Kröber, M., Bekel, T., Diaz, N. N., Goesmann, A., Jaenicke, S., Krause, L., et al. (2009). Phylogenetic characterization of a biogas plant microbial community integrating clone library 16S-rDNA sequences and metagenome sequence data obtained by 454-pyrosequencing. *J. Biotechnol.* 142, 38–49. doi: 10.1016/j.jbiotec.2009.02.010
- Kwietniewska, E., and Tys, J. (2014). Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation. *Renew. Sustain. Energy Rev.* 34, 491–500. doi: 10.1016/j.rser.2014.03.041
- Lakaniemi, A.-M., Hulatt, C. J., Thomas, D. N., Tuovinen, O. H., and Puhakka, J., a (2011). Biogenic hydrogen and methane production from *Chlorella vulgaris* and *Dunaliella tertiolecta* biomass. *Biotechnol. Biofuels* 4:34. doi: 10.1186/1754-6834-4-34
- Lam, M. K., and Lee, K. T. (2012). Microalgae biofuels: a critical review of issues, problems and the way forward. *Biotechnol. Adv.* 30, 673–690. doi: 10.1016/j.biotechadv.2011.11.008
- Langmead, B., and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359. doi: 10.1038/nmeth.1923
- Lassy, R. A., and Miller, C. G. (2000). Peptidase E, a peptidase specific for N-terminal aspartic dipeptides, is a serine hydrolase. *J. Bacteriol.* 182, 2536–2543. doi: 10.1128/JB.182.9.2536-2543.2000
- Lavrič, L., Cerar, A., Fanel, L., Lazar, B., Žitnik, M., and Logar, R. M. (2017). Thermal pretreatment and bioaugmentation improve methane yield of microalgal mix produced in thermophilic anaerobic digestate. *Anaerobe* 46, 162–169. doi: 10.1016/j.anaerobe.2017.02.001
- Lee, G. H., Rhee, M. S., Chang, D. H., Lee, J., Kim, S., Yoon, M. H., et al. (2013). *Oscillibacter ruminantium* sp. nov., isolated from the rumen of Korean native cattle. *Int. J. Syst. Evol. Microbiol.* 63, 1942–1946. doi: 10.1099/ijss.0.041749-0
- Leite, A. F., Janke, L., Lv, Z., Harms, H., Richnow, H. H., and Nikolausz, M. (2015). Improved monitoring of semi-continuous anaerobic digestion of sugarcane waste: effects of increasing organic loading rate on methanogenic community dynamics. *Int. J. Mol. Sci.* 16, 23210–23226. doi: 10.3390/ijms161023210
- Lemos, L. N., Pereira, R. V., Quaggio, R. B., Martins, L. F., Moura, L. M. S., da Silva, A. R., et al. (2017). Genome-centric analysis of a thermophilic and cellulolytic bacterial consortium derived from composting. *Front. Microbiol.* 8:644. doi: 10.3389/fmicb.2017.00644
- Li, C., Nges, I. A., Lu, W., and Wang, H. (2017). Assessment of the degradation efficiency of full-scale biogas plants: a comparative

- study of degradation indicators. *Bioresour. Technol.* 244, 304–312. doi: 10.1016/j.biortech.2017.07.157
- Li, D., Liu, C. M., Luo, R., Sadakane, K., and Lam, T. W. (2015). MEGAHit: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics* 31, 1674–1676. doi: 10.1093/bioinformatics/btv033
- Lienen, T., Kleyböcker, A., Brehmer, M., Kraume, M., Moeller, L., Görsch, K., et al. (2013). Floating layer formation, foaming, and microbial community structure change in full-scale biogas plant due to disruption of mixing and substrate overloading. *Energy Sustain. Soc.* 3:20. doi: 10.1186/2192-0567-3-20
- Lin, P. Y., Whang, L. M., Wu, Y. R., Ren, W. J., Hsiao, C. J., Li, S. L., et al. (2007). Biological hydrogen production of the genus *Clostridium*: metabolic study and mathematical model simulation. *Int. J. Hydrogen Energy* 32, 1728–1735. doi: 10.1016/j.ijhydene.2006.12.009
- Liu, C., Finegold, S. M., Song, Y., and Lawson, P. A. (2008). Reclassification of *Clostridium coccoides*, *Ruminococcus hansenii*, *Ruminococcus hydrogenotrophicus*, *Ruminococcus luti*, *Ruminococcus productus* and *Ruminococcus schinkii* as *Blautia coccoides* gen. nov., comb. nov., *Blautia hansenii* comb. nov., *Blautia hydroge notrophica* comb. nov., *Blautia luti* comb. nov., *Blautia producta* comb. nov., *Blautia schinkii* comb. nov. and description of *Blautia wexlerae* sp. nov., isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* 58, 1896–1902. doi: 10.1099/ijs.0.65208-0
- Liu, P., and Lu, Y. (2018). Concerted metabolic shifts give new insights into the syntrophic mechanism between propionate-fermenting *Pelotomaculum thermopropionicum* and hydrogenotrophic *Methanocella conradii*. *Front. Microbiol.* 9:1551. doi: 10.3389/fmicb.2018.01551
- Liu, T., Sun, L., Müller, B., and Schnürer, A. (2017). Importance of inoculum source and initial community structure for biogas production from agricultural substrates. *Bioresour. Technol.* 245, 768–777. doi: 10.1016/j.biortech.2017.08.213
- Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M., and Henrissat, B. (2014). The carbohydrate-active enzymes database (CAZy) in 2013. *Nucleic Acids Res.* 42, 490–495. doi: 10.1093/nar/gkt1178
- Lueders, T., Pommerenke, B., and Friedrich, M. W. (2004). Stable-isotope probing of microorganisms thriving at thermodynamic limits: syntrophic propionate oxidation in flooded soil. *Appl. Environ. Microbiol.* 70, 5778–5786. doi: 10.1128/AEM.70.10.5778-5786.2004
- Luton, P. E., Wayne, J. M., Sharp, R. J., and Riley, P. W. (2002). The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology* 148, 3521–3530. doi: 10.1099/00221287-148-11-3521
- Mahdy, A., Fotidis, I. A., Mancini, E., Ballesteros, M., González-Fernández, C., and Angelidaki, I. (2017). Ammonia tolerant inocula provide a good base for anaerobic digestion of microalgae in third generation biogas process. *Bioresour. Technol.* 225, 272–278. doi: 10.1016/j.biortech.2016.11.086
- Mahdy, A., Mendez, L., Ballesteros, M., and González-Fernández, C. (2015). Protease pretreated *Chlorella vulgaris* biomass bioconversion to methane via semi-continuous anaerobic digestion. *Fuel* 158, 35–41. doi: 10.1016/j.fuel.2015.04.052
- Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., et al. (2006). Comparative genomics of the lactic acid bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 103, 15611–15616. doi: 10.1073/pnas.0607117103
- Mao, C., Feng, Y., Wang, X., and Ren, G. (2015). Review on research achievements of biogas from anaerobic digestion. *Renew. Sustain. Energy Rev.* 45, 540–555. doi: 10.1016/j.rser.2015.02.032
- Martínez-Rodríguez, S., García-Pino, A., Heras-Vázquez, F. J., Las, Clemente-Jiménez, J. M., Rodríguez-Vico, F., García-Ruiz, J. M., et al. (2012). Mutational and structural analysis of l-N-carbamoylase reveals new insights into a peptidase M20/M25/M40 family member. *J. Bacteriol.* 194, 5759–5768. doi: 10.1128/JB.01056-12
- Matsui, H., Ogata, K., Tajima, K., Nakamura, M., Nagamine, T., Aminov, R. I., et al. (2000). Phenotypic characterization of polysaccharidases produced by four *Prevotella* type strains. *Curr. Microbiol.* 41, 45–49. doi: 10.1007/s002840010089
- Maus, I., Bremges, A., Stolze, Y., Hahnke, S., Cibis, K. G., Koeck, D. E., et al. (2017). Genomics and prevalence of bacterial and archaeal isolates from biogas-producing microbiomes. *Biotechnol. Biofuels* 10, 1–22. doi: 10.1186/s13068-017-0947-1
- Maus, I., Koeck, D. E., Cibis, K. G., Hahnke, S., Kim, Y. S., Langer, T., et al. (2016). Unraveling the microbiome of a thermophilic biogas plant by metagenome and metatranscriptome analysis complemented by characterization of bacterial and archaeal isolates. *Biotechnol. Biofuels* 9, 1–28. doi: 10.1186/s13068-016-0581-3
- Maus, I., Wibberg, D., Stantscheff, R., Eikmeyer, F. G., Seffner, A., Boelter, J., et al. (2012). Complete genome sequence of the hydrogenotrophic, methanogenic archaeon *Methanoculleus bourgensis* strain MS2T, isolated from a sewage sludge digester. *J. Bacteriol.* 194, 5487–5488. doi: 10.1128/JB.01292-12
- McInerney, M. J., Bryant, M. P., Hespell, R. B., and Costerton, J. W. (1981). *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, aytrophic, fatty acid-oxidizing bacterium. *Appl. Environ. Microbiol.* 41, 1029–1039.
- Menzel, P., Ng, K. L., and Krogh, A. (2016). Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nat. Commun.* 7:11257. doi: 10.1038/ncomms11257
- Müller, B., Sun, L., Westerholm, M., and Schnürer, A. (2016). Bacterial community composition and fhs profiles of low- and high-ammonia biogas digesters reveal novel syntrophic acetate-oxidising bacteria. *Biotechnol. Biofuels* 9:48. doi: 10.1186/s13068-016-0454-9
- Mussnug, J. H., Klassen, V., Schlüter, A., and Kruse, O. (2010). Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J. Biotechnol.* 150, 51–56. doi: 10.1016/j.jbiotec.2010.07.030
- Nagai, F., Watanabe, Y., and Morotomi, M. (2010). *Slackia piriformis* sp. nov. and *Collinsella tanakaei* sp. nov., new members of the family Coriobacteriaceae, isolated from human faeces. *Int. J. Syst. Evol. Microbiol.* 60, 2639–2646. doi: 10.1099/ijs.0.017533-0
- Nakajima, M., Tanaka, N., Furukawa, N., Nihira, T., Kodutsumi, Y., Takahashi, Y., et al. (2017). Mechanistic insight into the substrate specificity of 1,2-β-oligoglucan phosphorylase from *Lachnoclostridium phytofermentans*. *Sci. Rep.* 7:42671. doi: 10.1038/srep42671
- Narihiro, T., Nobu, M. K., Kim, N. K., Kamagata, Y., and Liu, W. T. (2015). The nexus of syntrophy-associated microbiota in anaerobic digestion revealed by long-term enrichment and community survey. *Environ. Microbiol.* 17, 1707–1720. doi: 10.1111/1462-2920.12616
- Nelson, M. C., Morrison, M., and Yu, Z. (2011). A meta-analysis of the microbial diversity observed in anaerobic digesters. *Bioresour. Technol.* 102, 3730–3739. doi: 10.1016/j.biortech.2010.11.119
- Neshat, S. A., Mohammadi, M., Najafpour, G. D., and Lahijani, P. (2017). Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production. *Renew. Sustain. Energy Rev.* 79, 308–322. doi: 10.1016/j.rser.2017.05.137
- Nettmann, E., Bergmann, I., Pramschüfer, S., Mundt, K., Plogsties, V., Herrmann, C., et al. (2010). Polyphasic analyses of methanogenic archaeal communities in agricultural biogas plants. *Appl. Environ. Microbiol.* 76, 2540–2548. doi: 10.1128/AEM.01423-09
- Nielsen, H. B., and Angelidaki, I. (2008). Strategies for optimizing recovery of the biogas process following ammonia inhibition. *Bioresour. Technol.* 99, 7995–8001. doi: 10.1016/j.biortech.2008.03.049
- Niu, Q., Kubota, K., Qiao, W., Jing, Z., Zhang, Y., and Yu-You, L. (2015). Effect of ammonia inhibition on microbial community dynamic and process functional resilience in mesophilic methane fermentation of chicken manure. *J. Chem. Technol. Biotechnol.* 90, 2161–2169. doi: 10.1002/jctb.4527
- Nobu, M. K., Narihiro, T., Rinke, C., Kamagata, Y., Tringe, S. G., Woyke, T., et al. (2015). Microbial dark matter ecogenomics reveals complex synergistic networks in a methanogenic bioreactor. *ISME J.* 9, 1710–1722. doi: 10.1038/ismej.2014.256
- Nolla-Ardévol, V., Peces, M., Strous, M., and Tegetmeyer, H. E. (2015a). Metagenome from a *Spirulina* digesting biogas reactor: analysis via binning of contigs and classification of short reads. *BMC Microbiol.* 15:277. doi: 10.1186/s12866-015-0615-1
- Nolla-Ardévol, V., Strous, M., and Tegetmeyer, H. E. (2015b). Anaerobic digestion of the microalga *Spirulina* at extreme alkaline conditions: biogas production, metagenome and metatranscriptome. *Front. Microbiol.* 6:597. doi: 10.3389/fmicb.2015.00597
- Nunoura, T., Oida, H., Miyazaki, J., Miyashita, A., Imachi, H., and Takai, K. (2008). Quantification of *mcrA* by fluorescent PCR in methanogenic and methanotrophic microbial communities. *FEMS Microbiol. Ecol.* 64, 240–247. doi: 10.1111/j.1574-6941.2008.00451.x

- Oulas, A., Pavlouidi, C., Polymenakou, P., Pavlopoulos, G. A., Papanikolaou, N., Kotoulas, G., et al. (2015). Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. *Bioinform. Biol. Insights* 9, 75–88. doi: 10.4137/BBI.S12462
- Pap, B., Györkei, Á., Boboescu, I. Z., Nagy, I. K., Bíró, T., Kondorosi, É., et al. (2015). Temperature-dependent transformation of biogas-producing microbial communities points to the increased importance of hydrogenotrophic methanogenesis under thermophilic operation. *Bioresour. Technol.* 177, 375–380. doi: 10.1016/j.biortech.2014.11.021
- Parks, D. H., and Beiko, R. G. (2010). Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* 26, 715–721. doi: 10.1093/bioinformatics/btq041
- Passos, F., Cordeiro, P. H. M., Baeta, B. E. L., de Aquino, S. F., and Perez-Elvira, S. I. (2018). Anaerobic co-digestion of coffee husks and microalgal biomass after thermal hydrolysis. *Bioresour. Technol.* 253, 49–54. doi: 10.1016/j.biortech.2017.12.071
- Passos, F., Solé, M., Garcia, J., and Ferrer, I. (2013). Biogas production from microalgae grown in wastewater: effect of microwave pretreatment. *Appl. Energy* 108, 168–175. doi: 10.1016/j.apenergy.2013.02.042
- Passos, F., Uggetti, E., Carrère, H., and Ferrer, I. (2014). Pretreatment of microalgae to improve biogas production: a review. *Bioresour. Technol.* 172, 403–412. doi: 10.1016/j.biortech.2014.08.114
- Pelletier, E., Kreimeyer, A., Bocs, S., Rouy, Z., Gyapay, G., Chouari, R., et al. (2008). “*Candidatus Cloacamonas acidaminovorans*”: genome sequence reconstruction provides a first glimpse of a new bacterial division. *J. Bacteriol.* 190, 2572–2579. doi: 10.1128/JB.01248-07
- Pester, M., Brambilla, E., Alazard, D., Rattei, T., Weinmaier, T., Han, J., et al. (2012). Complete genome sequences of *Desulfosporosinus orientis* DSM765T, *Desulfosporosinus youngiae* DSM17734T, *Desulfosporosinus meridiei* DSM13257T, and *Desulfosporosinus acidiphilus* DSM22704T. *J. Bacteriol.* 194, 6300–6301. doi: 10.1128/JB.01392-12
- Pester, M., and Brune, A. (2006). Expression profiles of fhs (FTHFS) genes support the hypothesis that spirochaetes dominate reductive acetogenesis in the hindgut of lower termites. *Environ. Microbiol.* 8, 1261–1270. doi: 10.1111/j.1462-2920.2006.01020.x
- Pobeheim, H., Munk, B., Müller, H., Berg, G., and Guebitz, G. M. (2010). Characterization of an anaerobic population digesting a model substrate for maize in the presence of trace metals. *Chemosphere* 80, 829–836. doi: 10.1016/j.chemosphere.2010.06.011
- Pope, P. B., Vivekanand, V., Eijnsink, V. G. H., and Horn, S. J. (2013). Microbial community structure in a biogas digester utilizing the marine energy crop *Saccharina latissima*. *3 Biotech* 3, 407–414. doi: 10.1007/s13205-012-0097-x
- Popp, D., Plugge, M. C., Kleinsteuber, S., Harms, H., and Strauber, H. (2017). Inhibitory effect of coumarin on syntrophic fatty acid-oxidizing and methanogenic cultures and biogas reactor microbiomes. *Appl. Environ. Microbiol.* 83, 1–14. doi: 10.1128/AEM.00438-17
- Posadas, E., García-Encina, P. A., Domínguez, A., Díaz, I., Becares, E., Blanco, S., et al. (2014). Enclosed tubular and open algal-bacterial biofilm photobioreactors for carbon and nutrient removal from domestic wastewater. *Ecol. Eng.* 67, 156–164. doi: 10.1016/j.ecoleng.2014.03.007
- Posten, C., and Schaub, G. (2009). Microalgae and terrestrial biomass as source for fuels-A process view. *J. Biotechnol.* 142, 64–69. doi: 10.1016/j.jbiotec.2009.03.015
- Purushe, J., Fouts, D. E., Morrison, M., White, B. A., Mackie, R. I., Coutinho, P. M., et al. (2010). Comparative genome analysis of *Prevotella ruminicola* and *Prevotella bryantii*: insights into their environmental niche. *Microb. Ecol.* 60, 721–729. doi: 10.1007/s00248-010-9692-8
- Ragsdale, S. W., and Pierce, E. (2009). Acetogenesis and the wood-ljungdahl pathway of CO<sub>2</sub> fixation. *Biochim. Biophys. Acta* 1784, 1873–1898. doi: 10.1016/j.bbapap.2008.08.012
- Rajagopal, R., Massé, D. I., and Singh, G. (2013). A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresour. Technol.* 143, 632–641. doi: 10.1016/j.biortech.2013.06.030
- Ravachol, J., Borne, R., Meynial-Salles, L., Soucaille, P., Pagès, S., Tardif, C., et al. (2015). Combining free and aggregated cellulolytic systems in the cellulosome-producing bacterium *Ruminiclostridium cellulolyticum*. *Biotechnol. Biofuels* 8:114. doi: 10.1186/s13068-015-0301-4
- Rawlings, N. D., Barrett, A. J., Thomas, P. D., Huang, X., Bateman, A., and Finn, R. D. (2018). The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. *Nucleic Acids Res.* 46, D624–D632. doi: 10.1093/nar/gkx1134
- Rehl, T., and Müller, J. (2011). Life cycle assessment of biogas digester processing technologies. *Resour. Conserv. Recycl.* 56, 92–104. doi: 10.1016/j.resconrec.2011.08.007
- Rétfalvi, T., Szabó, P., Hájos, A. T., Albert, L., Kovács, A., Milics, G., et al. (2016). Effect of co-substrate feeding on methane yield of anaerobic digestion of *Chlorella vulgaris*. *J. Appl. Phycol.* 28, 2741–2752. doi: 10.1007/s10811-016-0796-5
- Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N. N., Anderson, I. J., Cheng, J. F., et al. (2013). Insights into the phylogeny and coding potential of microbial dark matter. *Nature* 499, 431–437. doi: 10.1038/nature12352
- Rivière, D., Desvignes, V., Pelletier, E., Chaussonnerie, S., Guermazi, S., Weissenbach, J., et al. (2009). Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *ISME J.* 3, 700–714. doi: 10.1038/ismej.2009.2
- Rui, J., Li, J., Zhang, S., Yan, X., Wang, Y., and Li, X. (2015). The core populations and co-occurrence patterns of prokaryotic communities in household biogas digesters. *Biotechnol. Biofuels* 8, 1–15. doi: 10.1186/s13068-015-0339-3
- Schären, M., Kiri, K., Riede, S., Gardener, M., Meyer, U., Hummel, J., et al. (2017). Alterations in the rumen liquid-, particle- and epithelium-associated microbiota of dairy cows during the transition from a silage- and concentrate-based ration to pasture in spring. *Front. Microbiol.* 8:744. doi: 10.3389/fmicb.2017.00744
- Schenk, P. M., Thomas-Hall, S. R., Stephens, E., Marx, U. C., Mussnug, J. H., Posten, C., et al. (2008). Second generation biofuels: high-efficiency microalgae for biodiesel production. *BioEnergy Res.* 1, 20–43. doi: 10.1007/s12155-008-9008-8
- Schlüter, A., Bekel, T., Diaz, N. N., Dondrup, M., Eichenlaub, R., Gartemann, K. H., et al. (2008). The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology. *J. Biotechnol.* 136, 77–90. doi: 10.1016/j.jbiotec.2008.05.008
- Shida, G. M., Sader, L. T., Cavalcante De Amorim, E. L., Sakamoto, I. K., Maintinguer, S. I., Saavedra, N. K., et al. (2012). Performance and composition of bacterial communities in anaerobic fluidized bed reactors for hydrogen production: effects of organic loading rate and alkalinity. *Int. J. Hydrogen Energy* 37, 16925–16934. doi: 10.1016/j.ijhydene.2012.08.140
- Siegert, I., and Banks, C. (2005). The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochem.* 40, 3412–3418. doi: 10.1016/j.procbio.2005.01.025
- Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., and Zdobnov, E. M. (2015). BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31, 3210–3212. doi: 10.1093/bioinformatics/btv351
- Smith, K. S., and Ingram-Smith, C. (2007). Methanosaeta, the forgotten methanogen? *Trends Microbiol.* 15, 150–155. doi: 10.1016/j.tim.2007.02.002
- Solden, L., Lloyd, K., and Wrighton, K. (2016). The bright side of microbial dark matter: lessons learned from the uncultivated majority. *Curr. Opin. Microbiol.* 31, 217–226. doi: 10.1016/j.mib.2016.04.020
- Stolze, Y., Bremges, A., Rummig, M., Henke, C., Maus, I., Pühler, A., et al. (2016). Identification and genome reconstruction of abundant distinct taxa in microbiomes from one thermophilic and three mesophilic production-scale biogas plants. *Biotechnol. Biofuels* 9:156. doi: 10.1186/s13068-016-0565-3
- Sun, L., Liu, T., Müller, B., and Schnürer, A. (2016). The microbial community structure in industrial biogas plants influences the degradation rate of straw and cellulose in batch tests. *Biotechnol. Biofuels* 9:128. doi: 10.1186/s13068-016-0543-9
- Sundh, I., Carlsson, H., Nordberg, Å., Hansson, M., and Mathisen, B. (2003). Effects of glucose overloading on microbial community structure and biogas production in a laboratory-scale anaerobic digester. *Bioresour. Technol.* 89, 237–243. doi: 10.1016/S0960-8524(03)00075-0
- Szuhaj, M., Ács, N., Tengölics, R., Bodor, A., Rákhely, G., Kovács, K. L., et al. (2016). Conversion of H<sub>2</sub> and CO<sub>2</sub> to CH<sub>4</sub> and acetate in fed-batch biogas reactors by mixed biogas community: a novel route for the power-to-gas concept. *Biotechnol. Biofuels* 9:102. doi: 10.1186/s13068-016-0515-0

- Tian, G., Zhang, W., Dong, M., Yang, B., Zhu, R., Yin, F., et al. (2017). Metabolic pathway analysis based on high-throughput sequencing in a batch biogas production process. *Energy* 139, 571–579. doi: 10.1016/j.energy.2017.08.003
- Treu, L., Kougias, P. G., Campanaro, S., Bassani, I., and Angelidaki, I. (2016). Deeper insight into the structure of the anaerobic digestion microbial community; The biogas microbiome database is expanded with 157 new genomes. *Bioresour. Technol.* 216, 260–266. doi: 10.1016/j.biortech.2016.05.081
- Ueki, A., Akasaka, H., Suzuki, D., and Ueki, K. (2006). *Paludibacter propionigenes* gen. nov., sp. nov., a novel strictly anaerobic, Gram-negative, propionate-producing bacterium isolated from plant residue in irrigated rice-field soil in Japan. *Int. J. Syst. Evol. Microbiol.* 56, 39–44. doi: 10.1099/ijso.63896-0
- Uggetti, E., Passos, F., Solé, M., Garfi, M., and Ferrer, I. (2016). Recent achievements in the production of biogas from microalgae. *Waste Biomass Valor.* 8, 129–139. doi: 10.1007/s12649-016-9604-3
- Ummalyma, S. B., Gnansounou, E., Sukumaran, R. K., Sindhu, R., Pandey, A., and Sahoo, D. (2017). Biofloculation: an alternative strategy for harvesting of microalgae – An overview. *Bioresour. Technol.* 242, 227–235. doi: 10.1016/j.biortech.2017.02.097
- Vergara-Fernández, A., Vargas, G., Alarcón, N., and Velasco, A. (2008). Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. *Biomass Bioener.* 32, 338–344. doi: 10.1016/j.biombioe.2007.10.005
- Větrovský, T., and Baldrian, P. (2013). The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community Analyses. *PLoS ONE* 8:e57923. doi: 10.1371/journal.pone.0057923
- Wade, W. G. (2006). The genus *Eubacterium* and related genera. *Prokaryotes* 4, 823–835. doi: 10.1007/0-387-30744-3\_28
- Wang, W., Li, C., Li, F., Wang, X., Zhang, X., Liu, T., et al. (2016). Effects of early feeding on the host rumen transcriptome and bacterial diversity in lambs. *Sci. Rep.* 6:32479. doi: 10.1038/srep32479
- Wang, W., Xie, L., Luo, G., Zhou, Q., and Angelidaki, I. (2013). Performance and microbial community analysis of the anaerobic reactor with coke oven gas biomethanation and *in situ* biogas upgrading. *Bioresour. Technol.* 146, 234–239. doi: 10.1016/j.biortech.2013.07.049
- Wang, Y., Xu, L., Gu, Y. Q., and Coleman-Derr, D. (2016). MetaCoMET: a web platform for discovery and visualization of the core microbiome. *Bioinformatics* 32, 3469–3470. doi: 10.1093/bioinformatics/btw507
- Ward, A. J., Lewis, D. M., and Green, F. B. (2014). Anaerobic digestion of algae biomass: a review. *Algal Res.* 5, 204–214. doi: 10.1016/j.algal.2014.02.001
- Wei, Y., Zhou, H., Zhang, J., Zhang, L., Geng, A., Liu, F., et al. (2015). Insight into dominant cellulolytic bacteria from two biogas digesters and their glycoside hydrolase genes. *PLoS ONE* 10:e129921. doi: 10.1371/journal.pone.0129921
- Westerholm, M., Roos, S., and Schnürer, A. (2010). *Syntrophaceticus schinkii* gen. nov., sp. nov., an anaerobic, syntrophic acetate-oxidizing bacterium isolated from a mesophilic anaerobic filter. *FEMS Microbiol. Lett.* 309, 100–104. doi: 10.1111/j.1574-6968.2010.02023.x
- White, J. R., Nagarajan, N., and Pop, M. (2009). Statistical methods for detecting differentially abundant features in clinical metagenomic samples. *PLoS Comput. Biol.* 5:e1000352. doi: 10.1371/journal.pcbi.1000352
- Wirth, R., Kádár, G., Kakuk, B., Maróti, G., Bagi, Z., Szilágyi, Á., et al. (2018). The planktonic core microbiome and core functions in the cattle rumen by next generation sequencing. *Front. Microbiol.* 9:2285. doi: 10.3389/fmicb.2018.02285
- Wirth, R., Kovács, E., Maróti, G., Bagi, Z., Rákhely, G., and Kovács, K. L. (2012). Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. *Biotechnol. Biofuels* 5:41. doi: 10.1186/1754-6834-5-41
- Wirth, R., Lakatos, G., Böjti, T., Maróti, G., Bagi, Z., Kis, M., et al. (2015a). Metagenome changes in the mesophilic biogas-producing community during fermentation of the green alga *Scenedesmus obliquus*. *J. Biotechnol.* 215, 52–61. doi: 10.1016/j.jbiotec.2015.06.396
- Wirth, R., Lakatos, G., Maróti, G., Bagi, Z., Minárovics, J., Nagy, K., et al. (2015b). Exploitation of algal-bacterial associations in a two-stage biohydrogen and biogas generation process. *Biotechnol. Biofuels* 8:59. doi: 10.1186/s13068-015-0243-x
- Wu, Y. W., Simmons, B. A., and Singer, S. W. (2015). MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. *Bioinformatics* 32, 605–607. doi: 10.1093/bioinformatics/btv638
- Xia, L. C., Cram, J. A., Chen, T., Fuhrman, J. A., and Sun, F. (2011). Accurate genome relative abundance estimation based on shotgun metagenomic reads. *PLoS ONE* 6:e27992. doi: 10.1371/journal.pone.0027992
- Xu, K., Liu, H., Du, G., and Chen, J. (2009). Real-time PCR assays targeting formyltetrahydrofolate synthetase gene to enumerate acetogens in natural and engineered environments. *Anaerobe* 15, 204–213. doi: 10.1016/j.anaerobe.2009.03.005
- Yadvika, S., Sreekrishnan, T. R., Kohli, S., and Rana, V. (2004). Enhancement of biogas production from solid substrates using different techniques - A review. *Bioresour. Technol.* 95, 1–10. doi: 10.1016/j.biortech.2004.02.010
- Yan, X., Geng, A., Zhang, J., Wei, Y., Zhang, L., Qian, C., et al. (2013). Discovery of (hemi-) cellulase genes in a metagenomic library from a biogas digester using 454 pyrosequencing. *Appl. Microbiol. Biotechnol.* 97, 8173–8182. doi: 10.1007/s00253-013-4927-5
- Yen, H. W., and Brune, D. E. (2007). Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresour. Technol.* 98, 130–134. doi: 10.1016/j.biortech.2005.11.010
- Yen, H. W., Hu, I. C., Chen, C. Y., Ho, S. H., Lee, D. J., and Chang, J. S. (2013). Microalgae-based biorefinery - from biofuels to natural products. *Bioresour. Technol.* 135, 166–174. doi: 10.1016/j.biortech.2012.10.099
- Yenigün, O., and Demirel, B. (2013). Ammonia inhibition in anaerobic digestion: a review. *Process Biochem.* 48, 901–911. doi: 10.1016/j.procbio.2013.04.012
- Yoon, S. H., Ha, S., Lim, J., Kwon, S., and Chun, J. (2017). A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 110, 1281–1286. doi: 10.1007/s10482-017-0844-4
- Zamalloa, C., De Vrieze, J., Boon, N., and Verstraete, W. (2012). Anaerobic digestibility of marine microalgae *Phaeodactylum tricornutum* in a lab-scale anaerobic membrane bioreactor. *Appl. Microbiol. Biotechnol.* 93, 859–869. doi: 10.1007/s00253-011-3624-5
- Zhang, J., Mao, L., Zhang, L., Loh, K.-C., Dai, Y., and Tong, Y. W. (2017). Metagenomic insight into the microbial networks and metabolic mechanism in anaerobic digesters for food waste by incorporating activated carbon. *Sci. Rep.* 7:11293. doi: 10.1038/s41598-017-11826-5
- Zhilina, T. N., Appel, R., Probian, C., Brossa, E. L., Harder, J., Widdel, F., et al. (2004). *Alkaliflexus imshenetskii* gen. nov. sp. nov., a new alkaliphilic gliding carbohydrate-fermenting bacterium with propionate formation from a soda lake. *Arch. Microbiol.* 182, 244–253. doi: 10.1007/s00203-004-0722-0
- Zhu, X., Campanaro, S., Treu, L., Kougias, P. G., and Angelidaki, I. (2018). Novel ecological insights and functional roles during anaerobic digestion of saccharides unveiled by genome-centric metagenomics. *Water Res.* 151, 271–279. doi: 10.1016/j.watres.2018.12.041

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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