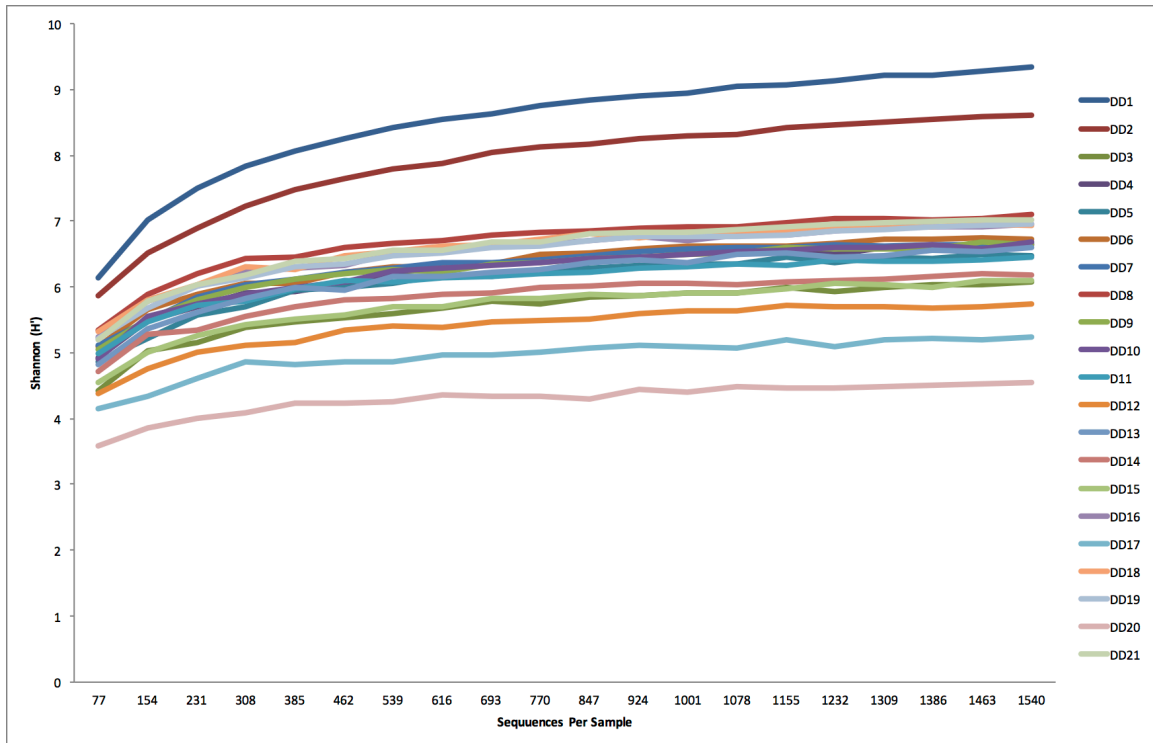


## Supplementary Material (two figures and six tables)

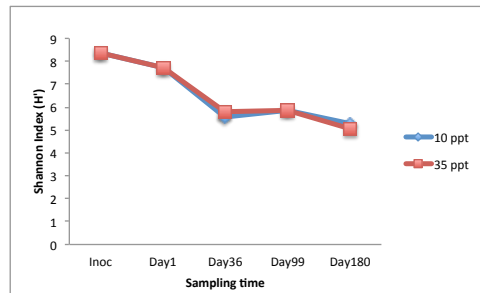
### Supplementary Figure 1 Rarefaction curves of community profile metagenomic datasets generated in this study

Sequencing details for each sample are listed in Supplementary Table 3.



## Supplementary Figure 2 Change in $\alpha$ diversity of the bioreactors over time

$\alpha$  diversity was measured using the unweighted unifrac measure, as described in Methods.



Change in alpha diversity over time

**Supplementary Table 1** Loading regime for 2L bioreactors

True OLR (g VS*L <sup>-1</sup> *d <sup>-1</sup> )	Specific volume of biogas produced (ml/g VS*d <sup>-1</sup> )  (1.0% salinity)	Specific volume of biogas produced (ml/g VS*d <sup>-1</sup> )  (3.5% salinity)	Ratio  (spec. biogas yield 35ppt/10 ppt)
1.4	98	55	0.56
1.7	132	79	0.60
3.5	112	49	0.44
4.6	75	25	0.33
5.7	45	45	1.00

**Supplementary Table 2** Peak specific yield and composition of biogas

Parameter	Value  1 % salinity	Value  3.5% salinity	Units
Peak specific yield of biogas	144	100	ml biogas per g VS*L <sup>-1</sup> *d <sup>-1</sup>
CH <sub>4</sub> / CO <sub>2</sub> ratio	1.17	1.07	dimensionless
Average % CH <sub>4</sub>	50.2%* (N=6;SE=2.1)	47.3% (N=6; SE=2.7)	vol. percent in biogas
Average % CO <sub>2</sub>	43.1%* (N=4;SE=4.9)	44.3% (N=5; SE=5.4)	vol. percent in biogas
Estimated % of total CO <sub>2</sub> in gas phase	86%	94%	assuming 1:1 stoichiometry of CO <sub>2</sub> :CH <sub>4</sub> production
Estimated % of total CO <sub>2</sub> in gas phase	56%	58%	Based on saturation solubility of CO <sub>2</sub> in seawater at 25°C and 1% or 3% salinity and no precipitation of carbonates
Estimated stoichiometry of CO <sub>2</sub> /CH <sub>4</sub>	1.5:1.0	1.6:1.0	Based on saturation solubility of CO <sub>2</sub> in seawater at 25°C and 1% or 3% salinity and no precipitation of carbonates

\* The sum of CH<sub>4</sub>+CO<sub>2</sub>+H<sub>2</sub>O was normalized to 95 % of the biogas in the 1.0 % salinity reactor (correction factor =0.83). No normalization was necessary for the 3.5 % salinity reactor

**Supplementary Table 3** Summary of information about each sample used in the  
16S rRNA community profile meta-analysis

Sample number	Sample ID	Substrate	True Name	Salinity	16S region	Description	References
1	H1	Sorghum	Sea sediment	High	V1-V3	Marine Sediment	Hollister et al. 2010
2	H2	Sorghum	Day 78	Low	V1-V3	Bioreactor	Hollister et al. 2010
3	H3	Sorghum	Day 30	Low	V1-V3	Bioreactor	Hollister et al. 2010
4	H4	Sorghum	Day 48	Low	V1-V3	Bioreactor	Hollister et al. 2010
5	H5	Sorghum	Day 62	Low	V1-V3	Bioreactor	Hollister et al. 2010
6	H6	Sorghum	Day 78	Low	V1-V3	Bioreactor	Hollister et al. 2010
7	DD1	Lagoon Sediment	To.Mar.13	Intermediate	V4	Lagoon Sediment	This work
8	DD2	Lagoon Sediment	T1.Apr.13	Intermediate	V4	Lagoon Sediment	This work
9	DD3	Sargassum	PD10.Jun.13	Low	V4	Bioreactor	This work
10	DD4	Sargassum	DG10.Jun.13	Low	V4	Bioreactor	This work
11	DD5	Sargassum	DG30.Jun.13	High	V4	Bioreactor	This work
12	DD6	Sargassum	PD30.Jun.13	High	V4	Bioreactor	This work
13	DD7	Sargassum	DG10.Aug.13	Low	V4	Bioreactor	This work
14	DD8	Sargassum	PD.30..Aug.13	High	V4	Bioreactor	This work
15	DD9	Sargassum	PD10.Aug.13	Low	V4	Bioreactor	This work
16	DD10	Sargassum	DG30.Aug.13	High	V4	Bioreactor	This work
17	DD11	Sargassum	PD30.Oct.13	High	V4	Bioreactor	This work
18	DD12	Sargassum	PD10.Oct.13	Low	V4	Bioreactor	This work
19	DD13	Sargassum	DG30.Oct.13	High	V4	Bioreactor	This work
20	DD14	Sargassum	DG10.Oct.13	Low	V4	Bioreactor	This work
21	DD15	Sargassum	S310.OCT.13	Low	V4	Bioreactor	This work
22	DD16	Sargassum	S3.35.OCT.13	High	V4	Bioreactor	This work
23	DD17	Sargassum	S1.10.OCT.13	Low	V4	Bioreactor	This work
24	DD18	Sargassum	S1.35.OCT.13	High	V4	Bioreactor	This work
25	DD19	Sargassum	PD.10ppt.M	Low	V4	Bioreactor	This work
26	DD20	Sargassum	DG.10ppt.M	Low	V4	Bioreactor	This work
27	DD21	Sargassum	PD.35ppt.M	High	V4	Bioreactor	This work
28	C1	Sewage Sludge	SS1as	Low	V3-V4	Bioreactor	Sundberg et al. 2013
29	C2	Sewage Sludge	SS1bs	Low	V3-V4	Bioreactor	Sundberg et al. 2013
30	C3	Sewage Sludge	SS3a	Low	V3-V4	Bioreactor	Sundberg et al. 2013
31	C4	Sewage Sludge	SS3b	Low	V3-V4	Bioreactor	Sundberg et al. 2013
32	C5	Sewage Sludge	SS4p	Low	V3-V4	Bioreactor	Sundberg et al. 2013
33	C6	Sewage Sludge	SS5	Low	V3-V4	Bioreactor	Sundberg et al. 2013
34	C7	Codigested Sludge	CD1	Low	V3-V4	Bioreactor	Sundberg et al. 2013

35	C8	Codigested Sludge	CD4ap	Low	V3-V4	Bioreactor	Sundberg et al. 2013
36	C9	Codigested Sludge	CD4bp	Low	V3-V4	Bioreactor	Sundberg et al. 2013
37	C10	Codigested Sludge	CD5ap	Low	V3-V4	Bioreactor	Sundberg et al. 2013
38	C11	Codigested Sludge	CD7a	Low	V3-V4	Bioreactor	Sundberg et al. 2013
39	BR.1	Sewage Sludge	NA	Low	V3-V5	Bioreactor	Shu et al. 2015
40	BR.2	Sewage Sludge	NA	Low	V3-V5	Bioreactor	Shu et al., 2015
41	BR.3	Sewage Sludge	NA	Low	V3-V5	Bioreactor	Shu et al., 2015
42	BR.4	Sewage Sludge	NA	Low	V3-V5	Bioreactor	Shu et al., 2015
43	BR.5	Sewage Sludge	NA	Low	V3-V5	Bioreactor	Shu et al., 2015
44	BR.6	Sewage Sludge	NA	Low	V3-V5	Bioreactor	Shu et al., 2015

Additional references:

Hollister et al. (2010) Microbial community composition and dynamics in a semi-industrial-scale facility operating under the MixAlco bioconversion platform. *Journal of Applied Microbiology* **110**, 587-596

Shu et al. (2015) Microbial structures and community functions of anaerobic sludge in six full-scale wastewater treatment plants as revealed by 454 high-throughput pyrosequencing. *Bioresource Technology* **186**, 163-172.

#### Supplementary Table 4 16S rRNA community profile datasets

In the table each sample is linked to its biomass feedstock, the number of sequences before and after filtering and the number of Operational Taxonomic Units (OTUs). A total of 324797 raw reads (sequence lengths (mean  $\pm$  std): 311.4244  $\pm$  169.7861) were used for the analysis. After filtering, a total 260877 sequences were used for the downstream analysis (sequence lengths (mean  $\pm$  std): 376.0740  $\pm$  144.5148)

Sample number	Sample ID	Substrate	Number of raw reads	Number of filtered reads	Number of OTUs	Proportion of archaea (%)
1	H1	Deep Sea sediment	7920	6958	3923	0
2	H2	Sorghum	4396	3940	1759	0
3	H3	Sorghum	10344	9736	3923	0
4	H4	Sorghum	4729	4402	1946	0
5	H5	Sorghum	4696	4386	2389	0
6	H6	Sorghum	4142	4076	2469	0
7	DD1	Lagoon Sediment	6092	4523	700	32.5
8	DD2	Lagoon Sediment	7821	5668	889	8.70
9	DD3	Sargassum	13359	10468	1566	0.83
10	DD4	Sargassum	11573	9597	1688	0.90
11	DD5	Sargassum	6458	5344	1044	0.72
12	DD6	Sargassum	9143	7044	1238	0.20
13	DD7	Sargassum	10576	8620	1317	0.50
14	DD8	Sargassum	10373	8085	1169	2.43
15	DD9	Sargassum	5008	4145	801	0.60
16	DD10	Sargassum	6446	5573	1000	0.68
17	DD11	Sargassum	8521	6571	940	0.05
18	DD12	Sargassum	9669	7963	1076	0.42
19	DD13	Sargassum	6446	6688	1208	0.36
20	DD14	Sargassum	3054	2548	538	0.28
21	DD15	Sargassum	6445	5943	978	1.05
22	DD16	Sargassum	9983	7798	1302	0.66
23	DD17	Sargassum	16963	14225	1321	0.48
24	DD18	Sargassum	13505	10587	1528	0.57
25	DD19	Sargassum	3294	2878	712	5.29
26	DD20	Sargassum	1731	1546	266	3.48

27	DD21	Sargassum	3399	3012	739	6.20
28	C1	Sewage Sludge	9085	6842	882	16.8
29	C2	Sewage Sludge	2166	1530	307	14.9
30	C3	Sewage Sludge	5956	4724	733	4.51
31	C4	Sewage Sludge	2127	1601	294	1.89
32	C5	Sewage Sludge	3288	2486	512	8.36
33	C6	Sewage Sludge	8838	4844	874	0.70
34	C7	Codigested Sludge	5523	2426	386	0.04
35	C8	Codigested Sludge	2776	1725	376	0.41
36	C9	Codigested Sludge	2588	1718	372	0.30
37	C10	Codigested Sludge	6446	1495	366	0
38	C11	Codigested Sludge	7316	4951	671	0.10
39	BR.1	Sewage Sludge	12077	11734	1318	3.98
40	BR.2	Sewage Sludge	14761	8066	1643	0.30
41	BR.3	Sewage Sludge	5111	4890	861	0.53
42	BR.4	Sewage Sludge	8189	7868	2199	0.02
43	BR.5	Sewage Sludge	11901	11415	2750	0
44	BR.6	Sewage Sludge	10563	10238	1520	0.26

**Supplementary Table 5** Physical parameters of the bioreactors associated with metagenomics samples

'PD' signifies pre-digester, 'DG' signifies main digester, 'NA' denotes not available.

Low salinity bioreactor

Sampling Day	pH		Alkalinity (g/L) CaCO <sub>3</sub> eq.		Total Solids (g/L)		Volatile Solids(g/L)	
	PD	DG	PD	DG	PD	DG	PD	DG
Day 1	7.30	7.30	0.72	0.72	NA	NA	NA	NA
Day 36	6.52	6.76	3.15	2.25	30.60	43.20	16.80	14.50
Day 99	7.22	7.30	3.62	4.34	66.50	74.00	20.20	14.40
Day 180	6.88	7.11	4.32	3.46	48.90	73.40	12.90	10.40

High salinity bioreactor

Sampling Day	PH		Alkalinity (g/L) CaCO <sub>3</sub> eq.		Total Solids (g/L)		Volatile Solids (g/L)	
	PD	DG	PD	DG	PD	DG	PD	DG
Day 1	7.33	7.33	0.72	0.72	NA	NA	NA	NA
Day 36	6.92	6.97	3.15	3.60	29.60	46.40	27.70	14.50
Day 99	7.20	6.98	8.68	3.62	33.20	56.90	14.40	14.40
Day 180	6.97	6.91	6.57	4.48	26.90	55.80	26.50	10.40



## Supplementary Table 6 Sequence statistics of shotgun metagenomic datasets

The table contains the information about the ten shotgun metagenomics data that were used for comparative meta-metabolic network and community profile analysis. The substrate type, number of raw reads, sequence length in base pairs (bp) and the number of 16S rRNA retrieved are given for each sample ID.

						Acetogenesis	Acidogenesis			Hydrogen Production				
Sample ID	Substrate	Number of raw reads	Sequence lengths (bp) (mean $\pm$ std)	16S rRNA	Proportion of archaeal 16S rDNA fragments ( %)	formyltetrahydrofolate synthetase genes(per 100000 predicted ORFs)	acetate kinase genes (per 100000 predicted ORFs)	butyrate kinase genes(per 100000 predicted ORFs)	butyryl-CoA:acetate-CoA transferase genes (per 100000 predicted ORFs)	[FeFe]-hydrogenase genes (per 100000 predicted ORFs)	[NiFe]-hydrogenase genes (per 100000 predicted ORFs)	mcrA genes (per 100000 predicted ORFs)	pfk genes (per 100000 predicted ORFs)	References
B1	Sargassum (high salt, 15 L bioreactor)	1635017	236 $\pm$ 43	1562	0.3	1.94	1.63	1.19	0.88	3.01	0.13	0.13	7.15	This work
B2	Sargassum (high salt, first stage, day 99)	2607391	284 $\pm$ 44	3214	9.02	4.92	3.54	1.94	6.49	10.41	0.63	4.59	6.72	This work
B3	Sargassum (high salt, second stage, day 99)	1544020	277 $\pm$ 56	1524	7.71	6.55	10.01	3.65	7.11	12.24	1.54	4.39	7.97	This work
B4	Panicum virgatum(Spirulina)	548733	524 $\pm$ 66	772	0	11.50	5.53	0.15	7.72	0.58	0.87	0.00	2.91	Allgaier et al. 2010
B5	Spirulina	1019333	159 $\pm$ 44	1862	30.33	26.48	7.33	3.98	2.20	7.53	0.00	1.18	3.43	Nolla-Ardèvol et al. 2015
B6	Codigested sludge	194460	201 $\pm$ 76	396	0	38.36	28.21	6.21	2.82	31.03	1.13	4.51	7.33	Wirth et al. 2015
B7	Sc obliquus	155532	195 $\pm$ 66	356	1.83	33.57	25.00	7.86	3.57	32.14	0.00	0.00	51.90	Wirth et al. 2015
B8	Codigested sludge	761308	213 $\pm$ 63	1131	2.25	38.71	11.31	8.31	6.13	15.26	0.55	0.00	19.11	Wirth et al. 2015
B9	Codigested sludge	781297	420 $\pm$ 40	1162	2.01	49.64	22.93	13.63	12.01	24.77	0.97	0.87	16.10	Li et al. 2013
B10	Codigested sludge	551339	358 $\pm$ 111	835	0.18	65.87	11.86	13.34	14.49	24.87	1.15	1.61	26.70	Li et al. 2013

Additional references:

Allgaier, M. et al. (2010) Targeted discovery of glycoside hydrolases from a switchgrass-adapted compost community. *PLoS ONE* **5**, e8812.

Nolla-Ardèvol et al. (2015) Metagenome from a Spirulina digesting biogas reactor: analysis via binning of contigs and classification of short reads. *BMC Microbiology* **15**, 277.

Wirth, R. et al. (2015) Metagenome changes in the mesophilic biogas-producing community during fermentation of the green alga *Scenedesmus obliquus*. *Journal of Biotechnology* **215**, 52-61.