



ARC Training Centre for the Transformation of Australia's Biosolids Resource

Industry Report:

Summary of microbial diversity in Australian anaerobic digesters.

From the global MiDAS database of microbes in anaerobic digesters.

Contact details:

Dr Christian Krohn^a - Biosolids Training Centre Postdoc

A/Prof. Morten K.D. Dueholm^b - Global MiDAS team leader

Prof. Damien J. Batstone^{a,c}, Theme Leader and MiDAS coordinator Australia

Prof. Andrew S. Ball, Biosolids Training Centre Director ^a

^a ARC Training Centre for the Transformation of Australia's Biosolids Resource,
RMIT University, Building 215, Level 3, Room 003-06, RMIT Bundoora West Campus, 225-245 Plenty
Road, Bundoora, Victoria 3083, Australia

^b Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University,
Aalborg, Denmark

^c Australian Centre for Water and Environmental Biotechnology, The University of Queensland,
Brisbane, Queensland, 4072, Australia

TABLE OF CONTENT

DEFINITIONS	2
INTRODUCTION	2
SUMMARY OF RESULTS	3
OVERVIEW.....	3
CORE ORGANISMS.....	3
DIVERSITY.....	4
METHANOGENS.....	4
FILAMENTOUS ORGANISMS	5
REFERENCES	14

Definitions

- V1-V3*: A PCR primer for targeting a variable region (V1-V3 region) in the 16S rRNA gene that represents bacterial organisms, especially in aerated systems. It is commonly used for sequencing sludge DNA and quantify bacterial organism, and part of the protocol at the Centre for Microbial Communities (CMC) in Aalborg Denmark for its wastewater monitoring programs of Danish treatment plants.
- V4*: A PCR primer for targeting a variable region (V4 region) in the 16S rRNA gene that represents bacterial and archaeal organisms and therefore includes methanogens. Archaea are important for anaerobic sludges as their presence is associated with biogas quality. This PCR primer was recently recommended by the CMC for anaerobic digesters, because it saves time and costs by covering both, bacteria and archaea.
- V3-V5*: A PCR primer for targeting a variable region (V3-V5 region) in the 16S rRNA gene that represents archaeal organisms, including methanogens. This PCR primer was commonly used to capture methanogens, but is superseded by the use of the V4 primer for anaerobic digesters.

Introduction

Optimising biological treatment trains at wastewater treatment plant (WWTPs) requires knowledge about the microbes involved in the treatment process. The Centre for Microbial Communities (CMC) at Aalborg University, Denmark, conducted a global survey of wastewater microbes, which included samples from more than 280 anaerobic digesters worldwide, including 38 anaerobic digesters from 15 participating treatment plants in Australia. The data was incorporated to expand the CMC's open-source database of wastewater microbes (called the MiDAS database) to version MiDAS 5.3 (Dueholm et al.,

2024). Along with this reference database, the CMC has developed an ecosystem-specific MiDAS taxonomy - a curated wastewater-specific taxonomy, enabling researchers worldwide to confidently link DNA sequences to microbial identities and most importantly compare their results to different studies across the world. The MiDAS database has become an invaluable resource for anyone wanting to study wastewater microbes and use them to optimise treatment trains and resource recoveries.

With support of Morten and the CMC, the Australian subset of this global dataset was analysed. This report provides a summary of the microbial diversity in 38 Australian anaerobic digesters, increasing our understanding about the microbes involved in the treatment process.

Summary of results

Overview

The microbial diversity of 38 anaerobic mesophilic digesters (a total of 67 samples including digester duplicates) located in 15 treatment plants, across five states (South Australia, Victoria, Queensland, New South Wales and Western Australia) was compared. A total of 2,735 individual genera were detected (V4 primers) with the majority of those classified/identified with the MiDAS taxonomy (Fig. 1). These genera consisted of 4,116 species (V4 primers) of which around 50% remained unclassified (Fig. 1).

Core organisms

Core organisms are those which are both broadly present (>20%) and with moderate-high abundance (>0.1%). As expected, only a small number of genera (223) were in this category in Australia, as anaerobic digestion is energetically constrained and specialised. These genera are likely most relevant for digestion performance, hence represent priority organisms for future functional characterisation. Fifty-three genera of those were shared by all digesters, plus another 49 genera were shared by all but one digester, which is the only thermal hydrolysis plant (Oxley Creek) (Fig. 2). The top 40 most abundant genera and phyla are compared in heatmaps (Figs. 3 and 4). With 54% of abundances (V4) Oxley Creek digesters contained the highest percentage of Firmicutes bacteria by a wide margin, with the second highest at 19% in Kawana. Firmicutes are a hydrolytic organism, and while there is significant functional redundancy here, microbiome is a major factor impacting degree of hydrolysis

and the effect of microbiome in hydrolytic efficiency is one of the major gaps in the link between AD microbiome and function (Krohn et al., 2022).

Diversity

Oxley Creek sludge contained the lowest diversity of all Australian plants (V4 and V3-V5 primer, Fig. 5). This is reasonable, as it is ammonia-constrained, and a thermal hydrolysis fed plant. Four of the seven plants with lowest microbial diversity, pre-treated their sludge, including Oxley Creek (THP), Malabar, North Head, and Bondi (recup thickening) (Fig. 5). The two plants containing the most diverse sludge microbes were Coombabah (WAS only) and Elanora in Queensland (Fig. 5), which did not pretreat the sludge. Generally, total diversity is a poor indicator of active diversity as dead microbes entering a reactor can artificially increase diversity (Krohn et al., 2024). Hence, in cases where sludge is pretreated such that dead biomass is degraded (e.g., thermal hydrolysis, Oxley Ck) it can be expected that ADs contain a lower but active diversity.

Compositional differences in sludge from each of the anaerobic digesters in Australia are represented as sample points in Principal Component Analysis (PCA) (Fig. 6). Each sample point represents the microbial composition of that sample, and the distance to other sample points indicates the degree to which the composition differed. For example, the microbial composition in digesters at Oxley Creek were clearly different to all other digesters, showing this treatment plant had a unique signature of both, bacteria (V1-V3) and archaea (V3-V5).

Furthermore, the microbial composition of each treatment plant was distinct to other plants. For example, the four digesters sampled at the Eastern Treatment Plant in Melbourne clustered closely together, as well as the four digesters sampled at Beenyup (V4, Fig. 6).

Methanogens

Methanogens are critical for biogas quality. *Methanothrix* (AKA *Methanosaetaceae*) species were the most abundant methanogens in all plants (43 – 63% of all archaea), indicative of methane production from acetate as the major pathway and a stable process state at time of sampling. *Methanosaeta* are an obligate acetate cleavage specialist, more sensitive to ammonia than other methanogens (Karakashev et al., 2005), and tend to operate with a very residual acetate level in comparison with other methanogens

(e.g., *Methanosarcina*, *Methanoculleus*, *Methanolinea*, *Methanobrevibacter*), which may operate at a higher ambient acetate level, or participate with bacterial partners in acetate oxidation rather than direct acetate cleavage (Karakashev et al., 2006). More information about *Methanotherix* can be found here: <https://www.midasfieldguide.com/guide/fieldguide/genus/methanotherix>.

For plants in Bondi, Malabar (New South Wales), Bolivar, Glenelg (South Australia) and Elanora (Queensland) (V3-V5, Fig. 5) the composition of methanogens was similar to each other. This is also seen in a heatmap, which shows that these plants contain a high abundance (21-27% of Archaea) of *Methanolinea* methanogens, distinct from all other plants (Fig. 7). *Methanolinea* cells produced methane from H₂/CO₂ and grow relatively well in acidic sludge compared to other methanogens (<https://www.midasfieldguide.com/guide/fieldguide/genus/methanolinea>). These same plants also contained the highest ratio of methanogens to bacteria (Fig. 8). Together with *Methanolinea*, these digesters also showed a distinct presence of Aminicenantales bacteria (midas_g_2538, Fig. 3). According to the MiDAS field guide (https://www.midasfieldguide.org/guide/fieldguide/genus/midas_g_2538) these bacteria are on average more abundant in manure and food waste digesters, compared to wastewater digesters globally. Not much is known about Aminicenantales bacteria yet, although both, *Methanolinea* and Aminicenantales bacteria have been associated with the degradation of aromatic compounds (Phan et al., 2021; Zheng et al., 2022). It highlighted that plants across different Australian states can have a similar composition of methanogens, which likely reflected selection pressures from AD operation and feedstocks.

Filamentous organisms

Known filamentous genera are shown in a heatmap (Fig. 9). *Candidatus Brevefilum* was present at significant abundances in Oxley Creek and Coombabah in Queensland. It is believed to grow well in ADs and was associated with foams (Jiang et al., 2021b, 2021a).

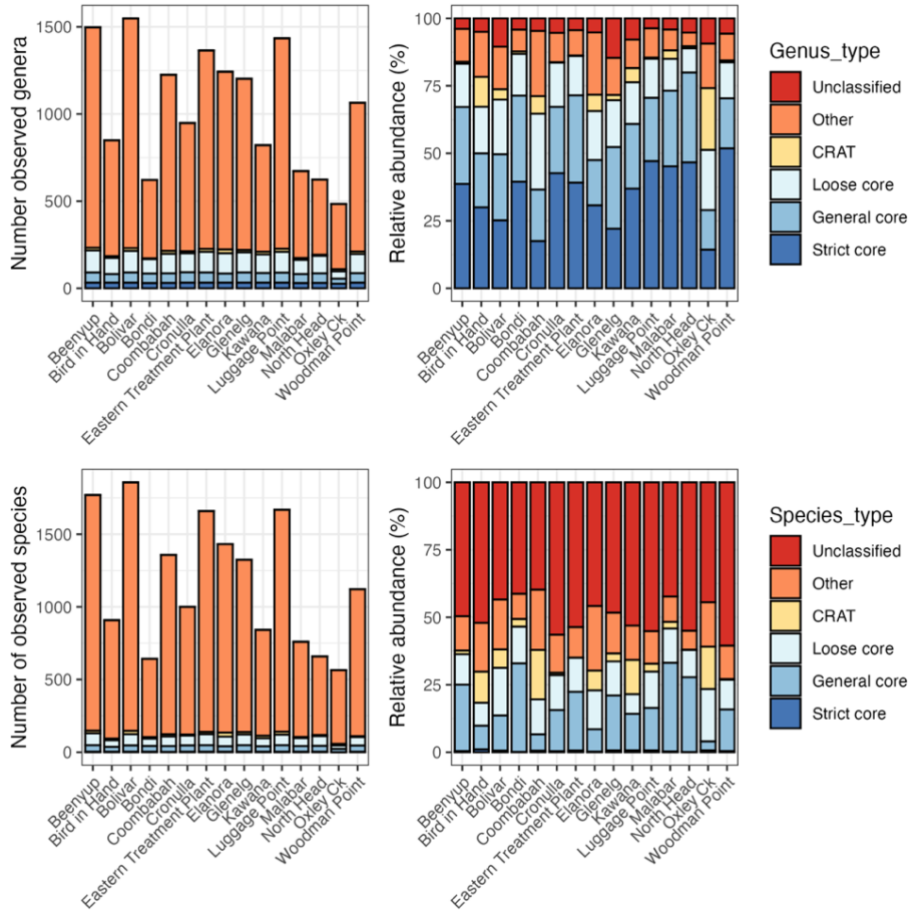


Figure 1. Number of observed genera (top) and species (bottom), respectively, and their relative abundance in Australian ADs (V4 primers). Values are divided into strict core, general core, loose core, CRAT, other taxa, and unclassified ASVs - calculated for Australian digesters only. The relative abundance of different groups was calculated based on the mean relative abundance of individual genera or species across samples. Definitions of strict core (>0.1% relative abundance in >80% of samples), general core (>0.1% relative abundance in >50% of samples), loose core (>0.1% relative abundance in >20% of samples), and CRAT (not part of the core, but present in at least one sample with a relative abundance >1%) were introduced in the MiDAS 4 release (Dueholm et al., 2022).

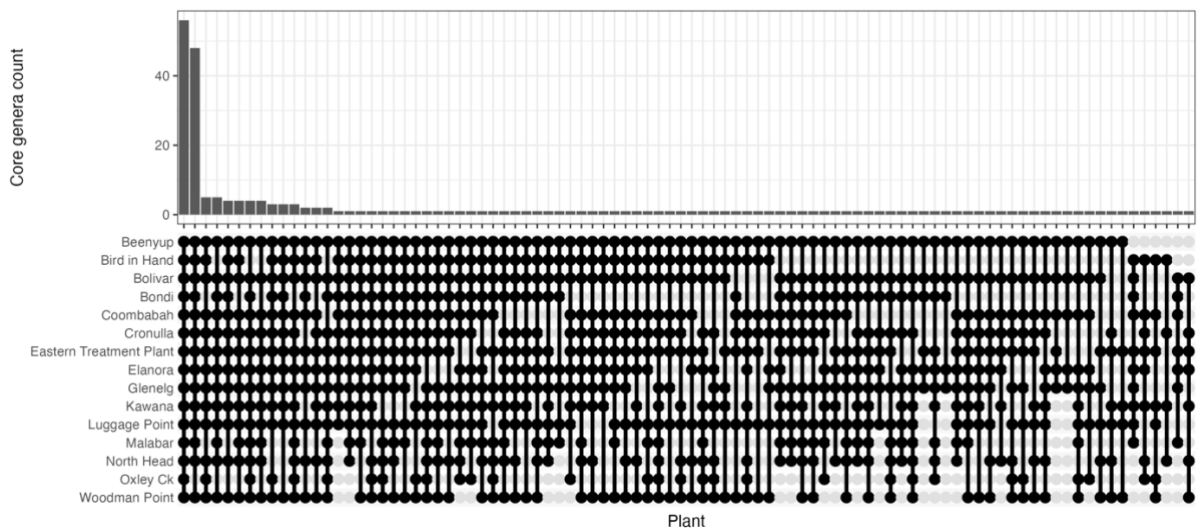


Figure 2. Number of genera (barplots at the top) and if they were present in different plants (represented by a dot at the bottom). Barplot shows the counts of 223 genera in total, which all represent core genera (loose, general and strict core genera). Bottom: The shared presence of core genera in Australian treatment plants is shown.

	NSW				QLD				SA			VIC	WA		
Unclassified; Unclassified	5	4	5	4	9	5	5	8	4	10	5	15	4	4	6
Cloacimonadaceae; Ca_Cloacimonas	12	3	8	1	0	4	0	15	7	2	0	0	4	9	23
Synergistaceae; Thermovirga	2	0	1	0	1	2	0	0	5	0	18	1	4	2	8
Spirochaetaceae; midas_g_1408	2	3	4	5	0	1	0	1	3	1	0	4	5	5	5
Rikenellaceae; DMER64	6	2	6	5	0	1	11	2	0	2	1	3	4	4	0
Cloacimonadaceae; W5	1	1	10	2	0	4	0	10	2	0	1	0	8	2	0
Lentimicrobiaceae; Lentimicrobium	3	1	2	2	4	1	1	3	6	1	2	1	4	1	1
Anaerolineaceae; midas_g_156	1	9	6	11	0	1	1	2	3	2	0	2	2	2	1
Bacteroidetes_vadinHA17; midas_g_19	1	0	2	0	0	1	1	0	6	3	1	1	2	1	1
Methanosaetaceae; Methanothrix	2	2	2	2	2	1	1	1	2	1	1	1	2	2	1
Smithellaceae; Smithella	1	2	2	3	0	1	2	2	3	1	1	1	2	2	1
Caldatribacteriaceae; Ca_Caldatribacterium	0	4	6	6	2	1	0	1	1	1	1	1	1	1	1
midas_f_1454; Exilispira	0	1	0	0	0	0	0	0	1	3	0	7	1	4	0
midas_f_13; midas_g_13	0	0	2	2	9	0	0	0	1	0	0	0	0	0	2
Fervidobacteriaceae; Fervidobacterium	1	2	1	2	0	0	0	0	1	0	0	1	3	2	3
Kosmotogaceae; Mesotoga	0	4	0	0	0	1	1	0	3	1	0	2	0	1	0
Prolixibacteraceae; midas_g_12	2	0	0	0	0	2	0	0	1	3	1	1	2	0	0
Petrogogaceae; SC103	0	0	0	0	0	9	4	0	2	0	0	0	0	0	0
Dysgonomonadaceae; Proteiniphilum	1	3	1	3	2	0	0	4	0	0	0	1	1	1	1
Petrogogaceae; AUTHM297	2	1	0	0	0	0	0	0	2	1	1	2	1	2	0
Anaerolineaceae; Flexilinea	1	1	4	4	0	0	1	1	0	0	0	1	1	2	0
Methanoregulaceae; Methanolinea	0	3	0	4	0	1	2	0	0	3	0	3	0	0	0
Ruminococcaceae; Ruminococcus	1	2	0	1	0	0	1	0	0	2	0	1	1	1	0
Methanospirillaceae; Methanospirillum	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1
Williamwhitmaniaceae; midas_g_141	0	0	0	0	2	1	0	0	1	0	4	0	1	1	2
Sedimentibacteraceae; Sedimentibacter	2	0	0	0	1	0	2	2	0	1	0	0	1	1	0
midas_f_55; midas_g_2538	0	5	0	3	0	0	0	0	0	2	0	2	0	1	1
midas_f_91940; midas_g_91940	2	0	2	0	1	0	0	0	1	0	0	0	2	0	1
Syntrophomonadaceae; Syntrophomonas	1	0	0	0	1	0	0	1	1	1	0	1	1	0	1
Anaerolineaceae; midas_g_789	0	0	0	0	0	0	0	0	0	5	0	4	0	0	0
Petrogogaceae; Defluviitoga	0	0	1	0	6	0	0	0	0	0	0	0	0	0	0
Syntrophorhabdaceae; Syntrophorhabdus	0	1	1	1	0	1	0	0	1	1	1	1	1	1	1
Methanomicrobiaceae; Methanoculleus	2	0	1	0	2	0	0	1	1	0	0	0	1	0	1
Christensenellaceae; Christensenellaceae_R-7_group	1	0	0	0	0	0	2	0	1	1	0	0	1	1	0
Synergistaceae; midas_g_249	1	1	2	1	0	0	1	2	0	0	0	0	1	1	0
Erysipelatoclostridiaceae; UCG-004	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
Ca_Phosphitivoracaceae; Ca_Phosphitivorax	0	1	0	1	1	0	1	0	1	1	0	1	0	0	1
Anaerolineaceae; Ca_Brevefilum	0	0	0	0	3	4	0	0	0	0	0	0	0	0	0
midas_f_76959; midas_g_76959	1	0	0	0	0	2	0	0	0	1	2	1	1	1	0
Methanobacteriaceae; Methanobacterium	0	3	2	2	0	0	0	0	0	0	0	0	1	0	0
Remaining taxa (2695)	42	36	27	34	47	55	59	40	39	47	58	41	39	45	36
	Cronulla -	Bondi -	North Head -	Malabar -	Oxley Ck -	Coombah -	Elanora -	Kawana -	Luggage Point -	Bolivar -	Bird in Hand -	Gleneilg -	Eastern Treatment Plant -	Beenyup -	Woodman Point -

Figure 3. Top 40 genera based on V4 amplicon data (archaea and bacteria) in Australian digesters. The percent abundance represents the mean abundance of each plant.

	NSW				QLD				SA			VIC	WA		
Bacteroidota-	22	13	16	15	14	19	27	17	22	18	24	14	21	18	11
Firmicutes-	14	6	9	9	54	4	15	19	8	11	6	6	9	7	12
Cloacimonadota-	13	4	18	4	0	8	2	24	9	2	2	0	11	11	23
Chloroflexi-	4	12	11	17	4	11	5	5	6	11	10	10	4	6	3
Unclassified-	5	4	5	4	9	5	5	8	4	10	5	15	4	4	6
Spirochaetota-	4	7	4	6	0	2	2	2	5	7	1	13	8	12	6
Synergistota-	5	2	5	4	1	3	2	4	7	2	19	2	7	5	11
Proteobacteria-	3	2	1	3	0	11	8	6	5	7	10	5	4	6	5
Thermotogota-	3	8	2	2	6	10	5	0	8	4	1	6	4	4	3
Halobacterota-	4	6	3	7	3	2	5	3	4	5	2	5	4	4	3
Verrucomicrobiota-	7	5	4	3	1	3	6	3	2	4	4	5	7	5	2
Desulfobacterota-	2	7	4	6	0	3	4	2	5	4	3	4	3	4	3
Acidobacteriota-	3	6	2	7	1	3	1	1	2	3	2	2	3	2	1
Planctomycetota-	2	2	1	1	1	4	3	1	2	2	3	2	2	3	1
Caldatribacteriota-	0	4	6	6	2	4	0	1	1	1	1	1	1	1	1
Euryarchaeota-	2	4	3	2	1	0	1	1	2	2	1	1	2	1	2
Actinobacteriota-	2	1	1	1	0	2	2	1	2	1	3	2	1	1	2
SAR324_cladeMarine_group_B-	0	1	0	1	1	0	1	0	1	1	0	1	0	0	1
Armatimonadota-	1	1	0	0	1	0	1	2	0	1	1	2	0	0	1
Hydrogenedentes-	1	0	1	0	0	0	0	1	1	0	1	0	1	1	1
Marinimicrobia_SAR406_clade-	0	2	1	0	0	0	0	0	0	0	0	0	1	1	0
Fibrobacterota-	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
Patescibacteria-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crenarchaeota-	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Sumerlaeota-	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Cyanobacteria-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myxococcota-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Campylobacterota-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thermoplasmata-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WPS-2-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bdellovibrionota-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrospirota-	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
WS1-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caldisericota-	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Coprothermobacterota-	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Dependentiae-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Elusimicrobiota-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fusobacteriota-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Latescibacterota-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nanoarchaeota-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Remaining taxa (18)-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Cronulla -	Bondi -	North Head -	Malabar -	Oxley Ck -	Coombah -	Elanora -	Kawana -	Luggage Point -	Bolivar -	Bird in Hand -	Glenelg -	Eastern Treatment Plant -	Beenyup -	Woodman Point -

Figure 4. Top 40 most abundance prokaryotes on phylum level based on the V4 primer.

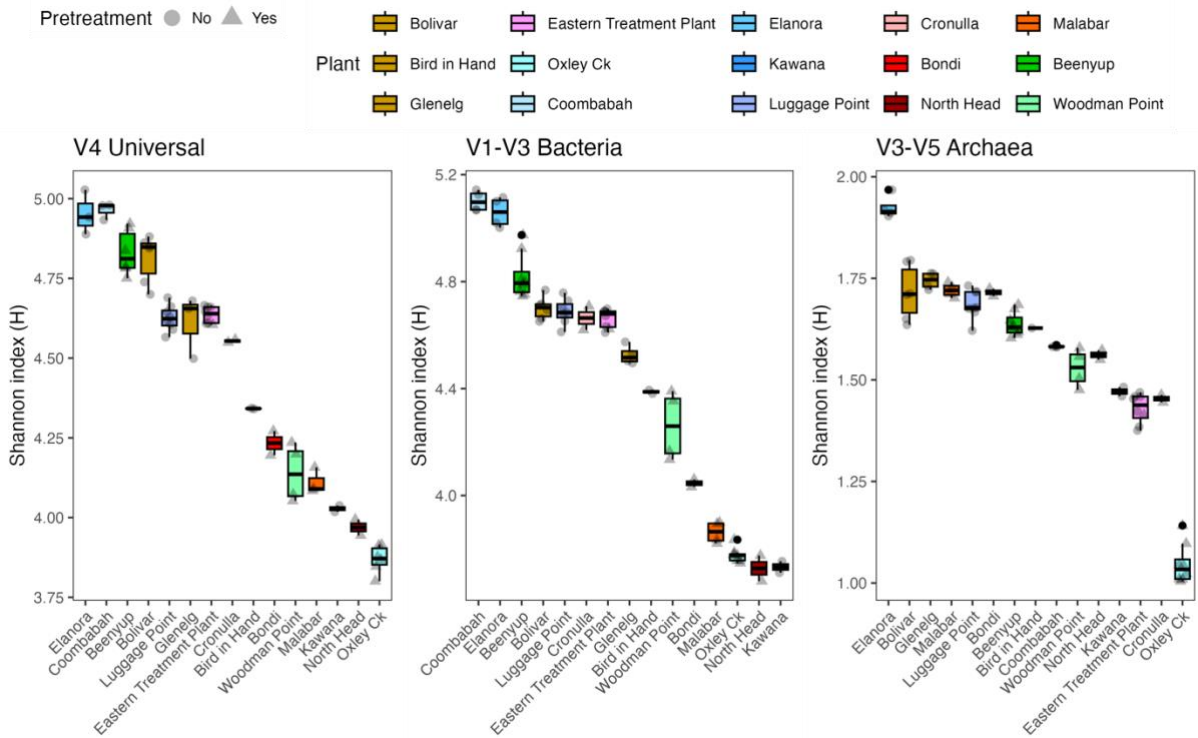


Figure 5. Shannon index (H') in decreasing order, based on three different primers used in this survey. The Shannon index reflects the diversity and distribution of non-rare organisms (also called the effective diversity). All reads were normalised (rarefied) prior to enumerating diversity indices.

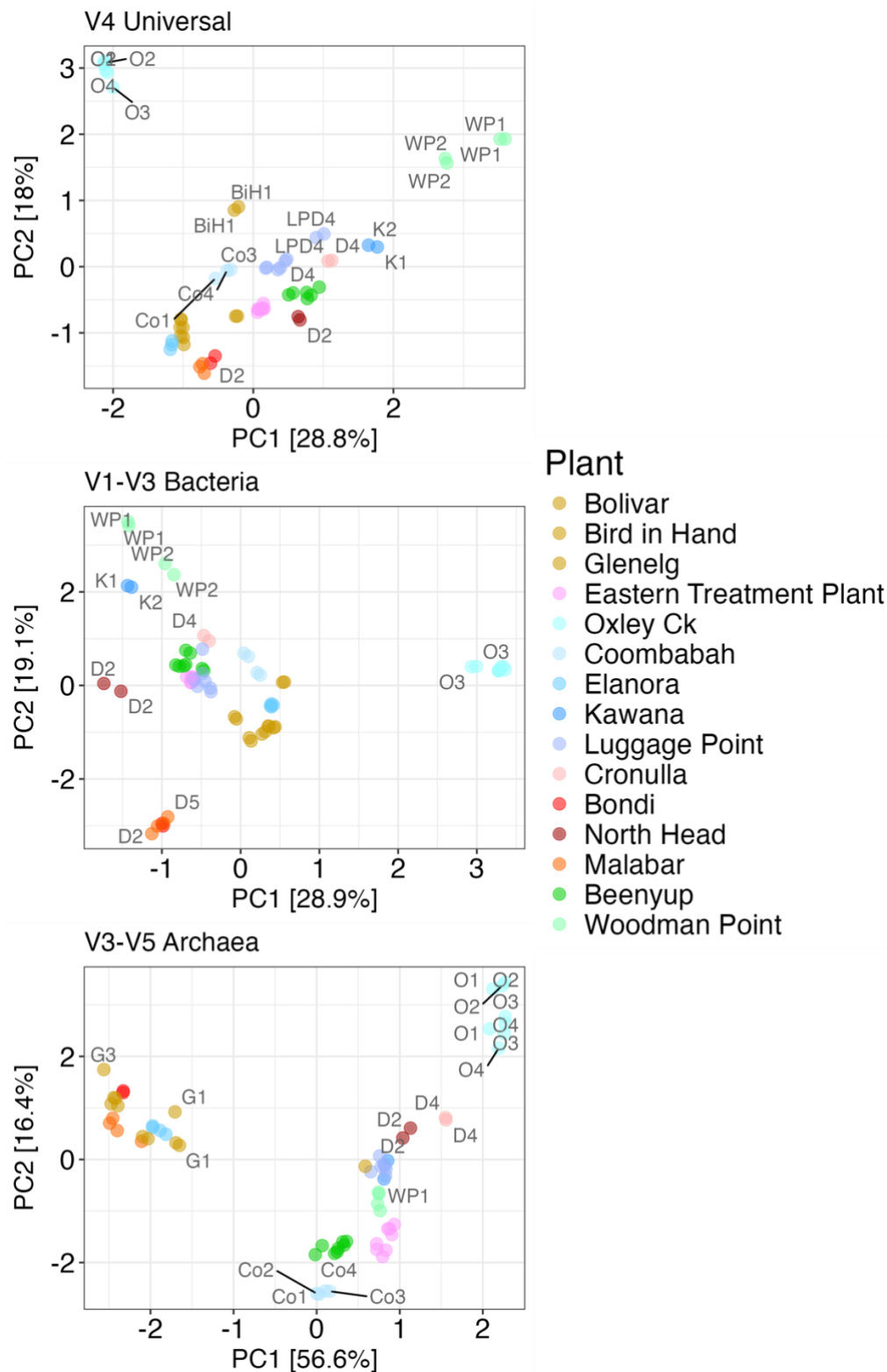


Figure 6. Compositional differences based on transformed abundances (centred-log transform) between Australian digesters in South Australia (beige), Victoria (pink), Queensland (blues), New South Wales (reds) and Western Australia (greens). Three different primers were used - V4 covering both bacteria and archaea, V1V3 covering bacteria and V3V5 covering archaea only. Each sample point represents the microbial composition of one digester. Some, but not all sample points are labelled with digester ID. No digester IDs were provided for Coombabah, Elanora and Kawana, hence their IDs are created randomly for the purpose of this figure. PC; principal component.

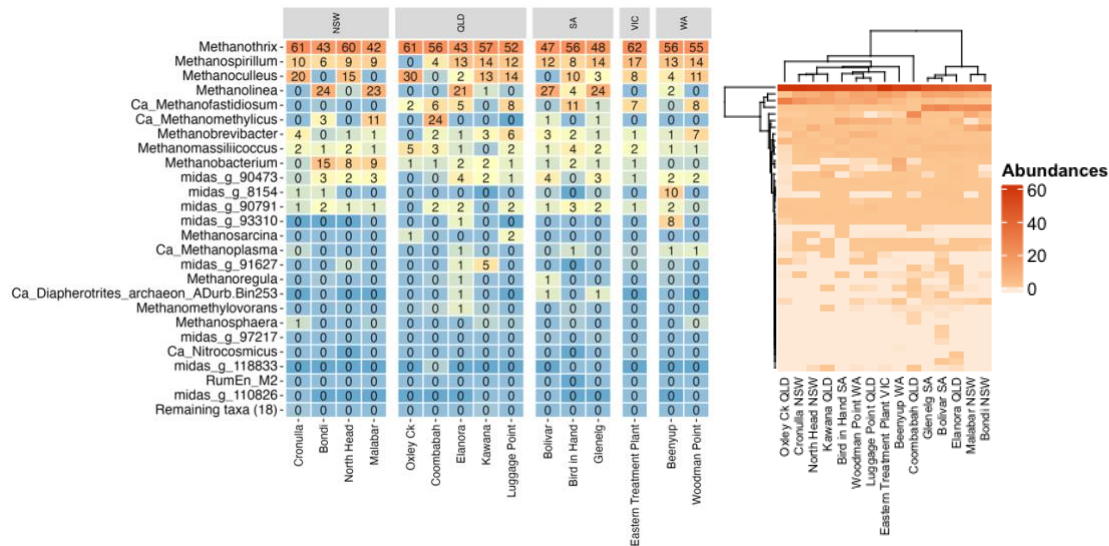


Figure 7. Left: Top 25 archaeal genera (methanogens) based on V3-V5 amplicon data in Australian digesters. The percent abundance represents the mean abundance relative to all archaea. Right: Clustering of treatment plants by similarity of archaeal composition (Ward’s minimum variance).

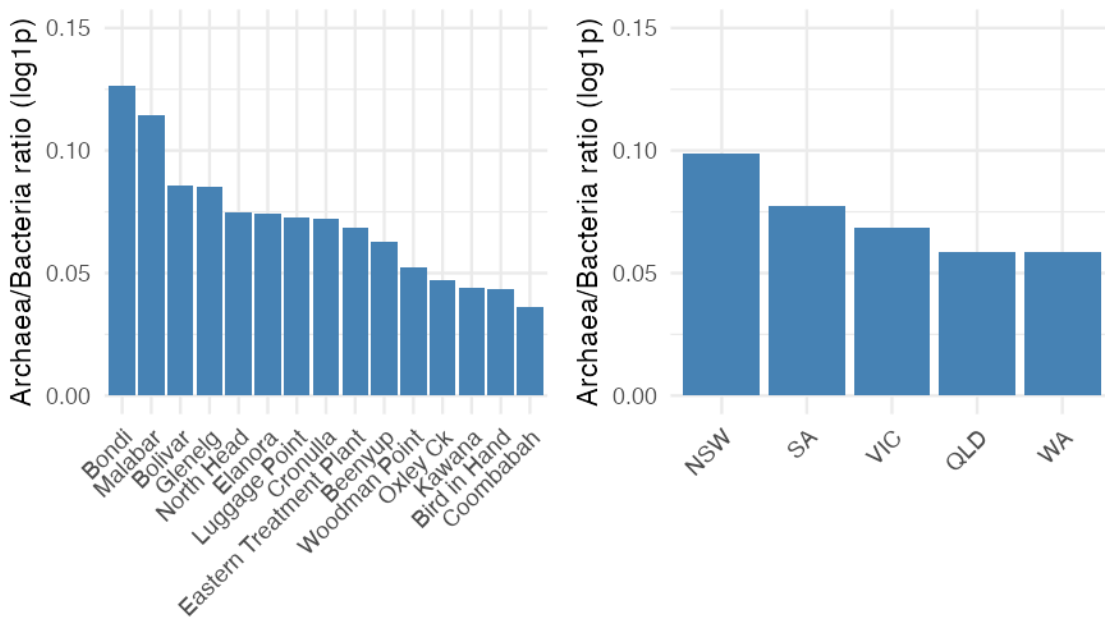
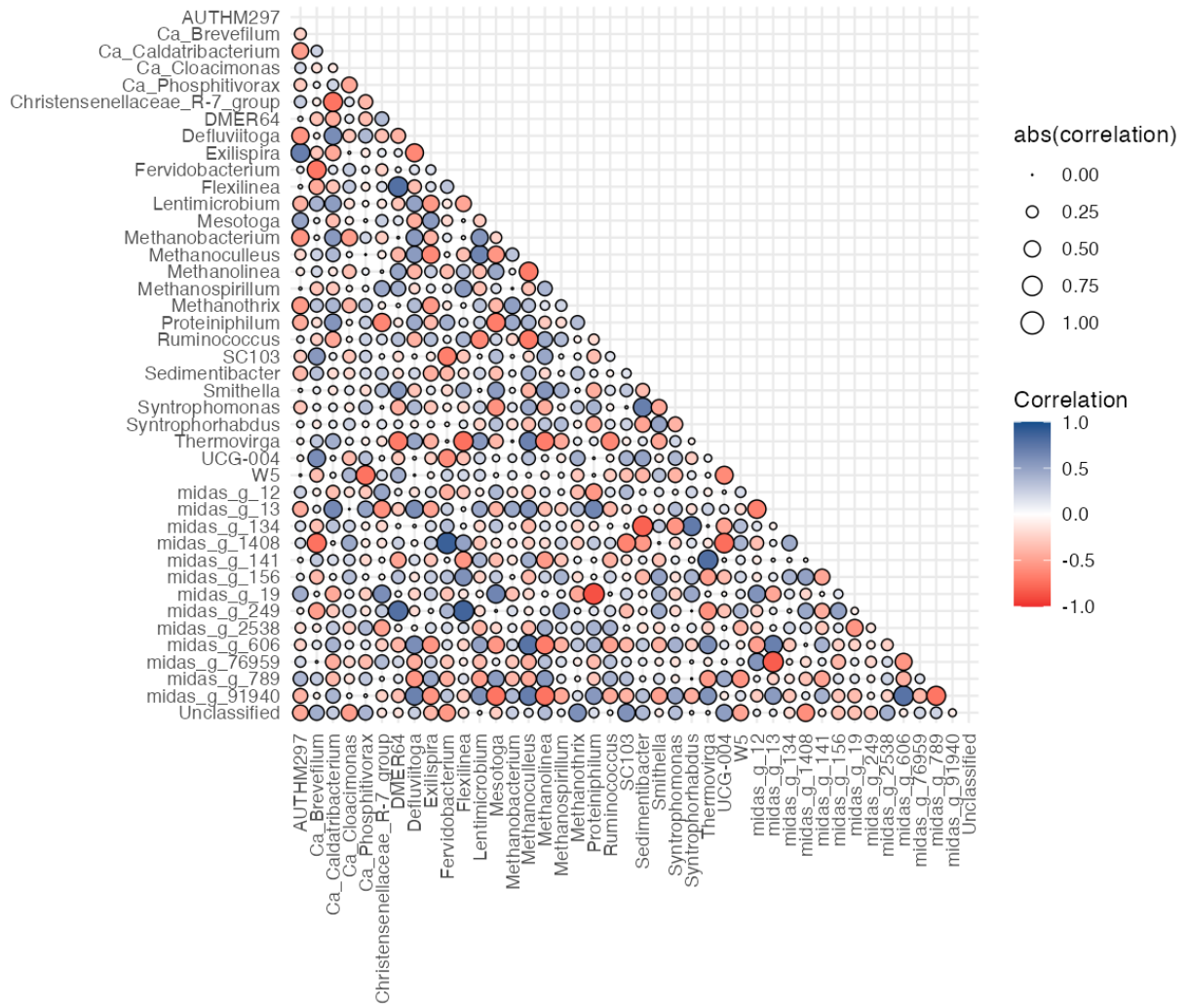


Figure 8. Archaeal load per plant and state. Calculated as the log ratio of the total relative abundances in percent of archaea and bacteria (V4 primers).

	NSW				QLD					SA			VIC	WA	
Anaerolineaceae; Ca_Brevefilum-	0	0.01	0	0	3.75	0.05	0.03	0.14	3.36	0.02	0.02	0	0	0.02	0
Mycobacteriaceae; Mycobacterium-	0.04	0.44	0.06	0.08	0.07	0.26	0.05	0.04	0	0.63	0.11	0.1	0.27	0.24	0.2
Microtrichaceae; IMCC26207-	0	0.16	0.01	0.03	0.18	0.07	0.08	0.27	0	0.22	0.15	0.08	0.06	0.03	0.14
Microtrichaceae; midas_g_5461-	0	0	0	0.01	0.07	0.25	0.01	0.11	0	0	0.04	0.11	0	0.21	0
Roseiflexaceae; Kouleothrix-	0	0	0	0	0.49	0	0	0.02	0	0.01	0.01	0.05	0.03	0.02	0.01
Ca_Promineofilaceae; Ca_Promineofilum-	0	0	0	0	0.12	0.09	0	0.09	0	0	0.02	0.01	0	0.06	0.06
Geminococcaceae; Ca_Alysiosphaera-	0	0	0	0	0.01	0	0	0	0	0.02	0.11	0.1	0.06	0.03	0.01
Amarolineaceae; Ca_Amarolinea-	0	0	0	0	0.53	0	0	0	0	0	0	0	0	0	0
Anaerolineaceae; Anaerolinea-	0.02	0.04	0.01	0.01	0.03	0.04	0.01	0.02	0	0.01	0.09	0.06	0.01	0.02	0.01
Nocardiaceae; Gordonia-	0.02	0.02	0.02	0.02	0.06	0.1	0.02	0.01	0	0.01	0.02	0.04	0.03	0.04	0.02
Caldilineaceae; midas_g_1668-	0	0.01	0	0	0.1	0.01	0	0.03	0	0.02	0.01	0.01	0.02	0.02	0.04
Microtrichaceae; midas_g_1605-	0	0	0	0	0.06	0.01	0	0.09	0	0	0	0	0	0	0
Carnobacteriaceae; Trichococcus-	0.03	0.01	0.02	0.01	0	0.01	0	0	0	0.01	0.01	0.04	0.03	0	0
Caldilineaceae; Ca_Caldilinea-	0	0	0	0	0	0	0	0.05	0	0	0	0.01	0	0.03	0
Thiotrichaceae; Thiothrix-	0	0	0	0	0.02	0	0.09	0.02	0	0	0	0	0	0	0
Nocardioidaceae; Nocardioides-	0	0.01	0	0	0.02	0.01	0	0.01	0	0	0.01	0	0	0	0.01
Saprosiraceae; Haliscomenobacter-	0	0	0	0	0.06	0.01	0	0	0	0	0	0	0	0	0
Microtrichaceae; midas_g_1877-	0	0.01	0	0	0.01	0.01	0	0.01	0	0.01	0	0	0	0	0
Nocardiaceae; Rhodococcus-	0	0.01	0.01	0	0	0.01	0	0	0	0	0	0	0	0	0
Comamonadaceae; Sphaerotilus-	0	0	0	0	0.02	0.01	0	0	0	0	0	0	0	0.01	0
Isosphaeraceae; Ca_Nostocoida-	0	0	0	0	0	0	0	0	0	0.02	0	0.01	0	0	0
Microtrichaceae; midas_g_120-	0	0	0	0	0.02	0.01	0	0	0	0	0	0	0	0	0.01
Comamonadaceae; Leptothrix-	0	0	0	0	0.01	0	0	0	0	0.01	0	0	0	0	0
Microtrichaceae; midas_g_17130-	0	0	0	0	0.01	0	0	0	0	0	0	0	0	0	0
Microtrichaceae; midas_g_10988-	0	0	0	0	0	0	0.02	0	0	0	0	0	0	0	0
Microtrichaceae; Ca_Microthrix-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nocardiaceae; Millisia-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Microtrichaceae; midas_g_3240-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nocardiaceae; Nocardia-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caldilineaceae; midas_g_344-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Bondi -	Cronulla -	Malabar -	North Head -	Coombah -	Elanora -	Kawana -	Luggage Point -	Oxley Ck -	Bird in Hand -	Bolivar -	Gleneig -	Eastern Treatment Plant -	Beenyup -	Woodman Point -

Figure 9. Relative abundances (%) of filamentous bacteria based on the V4 primer pair. The percent abundance represents the mean abundance relative to all bacteria and archaea.



Supplementary Figure S1. Pearson correlations of relative abundances (transformed into centred-log ratios) of the top 40 most abundance microbes (V4), across all Australian sludge samples.

References

- Dueholm, M.K.D., Andersen, K.S., Petersen, A.-K.C., Rudkjøbing, V., Alves, M., Bajón-Fernández, Y., Batstone, D., Butler, C., Cruz, M.C., Davidsson, Å., Erijman, L., Holliger, C., Koch, K., Kreuzinger, N., Lee, C., Lyberatos, G., Mutnuri, S., O'Flaherty, V., Oleskiewicz-Popiel, P., Pokorna, D., Rajal, V., Recktenwald, M., Rodríguez, J., Saikaly, P.E., Tooker, N., Vierheilig, J., Vrieze, J. De, Wurzbacher, C., Nielsen, P.H., 2024. MiDAS 5: Global diversity of bacteria and archaea in anaerobic digesters. *Nat. Commun.* 15, 5361. <https://doi.org/10.1038/s41467-024-49641-y>
- Dueholm, M.K.D., Nierychlo, M., Andersen, K.S., Rudkjøbing, V., Knutsson, S., Arriaga, S., Bakke, R., Boon, N., Bux, F., Christensson, M., Chua, A.S.M., Curtis, T.P., Cytryn, E., Erijman, L., Etchebehere, C., Fatta-Kassinos, D., Frigon, D., Garcia-Chaves, M.C., Gu, A.Z., Horn, H., Jenkins, D., Kreuzinger, N., Kumari, S., Lanham, A., Law, Y., Leiknes, T.O., Morgenroth, E., Muszyński, A., Petrovski, S., Pijuan, M., Pillai, S.B., Reis, M.A.M., Rong, Q., Rossetti, S., Seviour, R., Tooker, N., Vainio, P., van Loosdrecht, M., Vikraman, R., Wanner, J., Weissbrodt, D., Wen, X., Zhang, T., Nielsen, Per H., Albertsen, M., Nielsen, Per Halkjær, 2022. MiDAS 4: A global catalogue of full-length 16S rRNA gene sequences and taxonomy for studies of bacterial communities in wastewater treatment plants. *Nat. Commun.* 13, 1–15. <https://doi.org/10.1038/s41467-022-29438-7>
- Jiang, C., McIlroy, S.J., Qi, R., Petriglieri, F., Yashiro, E., Kondrotaitė, Z., Nielsen, P.H., 2021a. Identification of microorganisms responsible for foam formation in mesophilic anaerobic digesters treating surplus activated sludge. *Water Res.* 191, 116779. <https://doi.org/10.1016/j.watres.2020.116779>
- Jiang, C., Peces, M., Andersen, M.H., Kucheryavskiy, S., Nierychlo, M., Yashiro, E., Andersen, K.S., Kirkegaard, R.H., Hao, L., Høgh, J., Hansen, A.A., Dueholm, M.S., Nielsen, P.H., 2021b. Characterizing the growing microorganisms at species level in 46 anaerobic digesters at Danish wastewater treatment plants: A six-year survey on microbial community structure and key drivers. *Water Res.* 193, 116871. <https://doi.org/10.1016/j.watres.2021.116871>
- Karakashev, D., Batstone, D.J., Angelidaki, I., 2005. Influence of environmental conditions on methanogenic compositions in anaerobic biogas reactors. *Appl. Environ. Microbiol.* 71, 331–338. <https://doi.org/10.1128/AEM.71.1.331-338.2005>
- Karakashev, D., Batstone, D.J., Trably, E., Angelidaki, I., 2006. Acetate oxidation is the dominant methanogenic pathway from acetate in the absence of Methanosaetaceae. *Appl. Environ. Microbiol.* 72, 5138–5141.
- Krohn, C., Jansriphibul, K., Dias, D.A., Rees, C.A., Akker, B. van den, Boer, J.C., Plebanski, M., Surapaneni, A., O'Carroll, D., Richard, S., Batstone, D.J., Ball, A.S., 2024. Dead in the water – Role of relic DNA and primer choice for targeted sequencing surveys of anaerobic sewage sludge intended for biological monitoring. *Water Res.* 253, 121354. <https://doi.org/10.1016/j.watres.2024.121354>
- Krohn, C., Khudur, L., Dias, D.A., van den Akker, B., Rees, C.A., Crosbie, N.D., Surapaneni, A., O'Carroll, D.M., Stuetz, R.M., Batstone, D.J., Ball, A.S., 2022. The role of microbial ecology in improving the performance of anaerobic digestion of sewage sludge. *Front. Microbiol.* 13. <https://doi.org/10.3389/fmicb.2022.1079136>
- Phan, H. V., Kurisu, F., Kiba, K., Furumai, H., 2021. Optimized cultivation and syntrophic relationship of anaerobic benzene-degrading enrichment cultures under methanogenic conditions. *Microbes Environ.* 36, ME21028.
- Zheng, X., Zhao, Y., Xu, H., Fan, Q., Li, Y., Chen, L., Long, S., Angelidaki, I., Hua, D., 2022. Enhancing

the anaerobic digestion of papermaking black liquor with three-dimensional iron-carbon electrolysis and assessment of microbial community changes. *J. Environ. Chem. Eng.* 10, 108115.