Your microbes to action by 2027!

Working towards an Australia-wide decision-support tool for biological wastewater treatment processes

National Microbial Monitoring for Anaerobic Digesters and Activated Sludge Systems

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Industry partners: Seeking industry partners for 2027–2029 collaboration

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Transforming Biosolids

RMIT UNIVERSITY

Background

As Australia moves toward net zero, water utilities seek to better manage the microbial consortia that drive bioenergy recovery and mitigation of greenhouse-gas emissions during sewage treatment. For example, anaerobic digestion reduces sludge solids while producing renewable biogas, but its performance depends on a fragile microbial consortia that can fail without warning. Yet utilities lack direct ways to monitor this critical biomass. An economical biomass-to-screen monitoring and decision-support tool is urgently needed to ensure reliable, low-emission operation and guide troubleshooting and process optimisation.

Aims/Objectives

This project invites Australian wastewater treatment operators to join a national initiative to map and understand the microbial communities that underpin sewage treatment. Through coordinated sludge sampling and DNA sequencing, we will link key performance metrics — volatile solids destruction, methane yield, and nitrous oxide emissions — to the microbial consortia driving these processes. Participating operators will, for the first time, gain direct insight into their microbes, receiving weekly taxonomic profiles and tailored industry reports from anaerobic digesters and aeration tanks. This will provide unprecedented seasonal and spatial data on thousands of process-relevant bacteria and archaea, enabling informed, data-driven process optimisation.

Approach

- You send samples to RMIT Melbourne. We sequence them.
- Activated sludge and anaerobic digester sludge samples from 15 – 20 operators.
- Sampling protocols will be provided.
- Once per week over 1 year.
- Sludge stored at -80°C until DNA extraction and sequencing.
- A total of 2,000 sludge samples will be sequenced after collection is complete.
- We model your weekly performance data (VSD, gas flow, methane concentrations, feed sludge flow, sludge retention time…) to microbial abundances.
- Foaming and other operational events will be recorded and analysed in relation to microbial abundances.

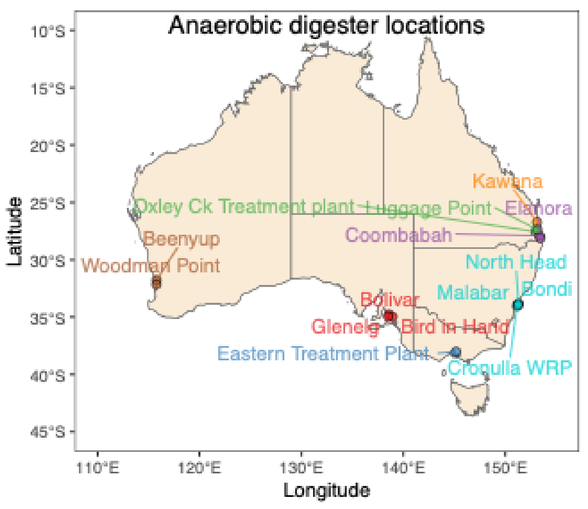
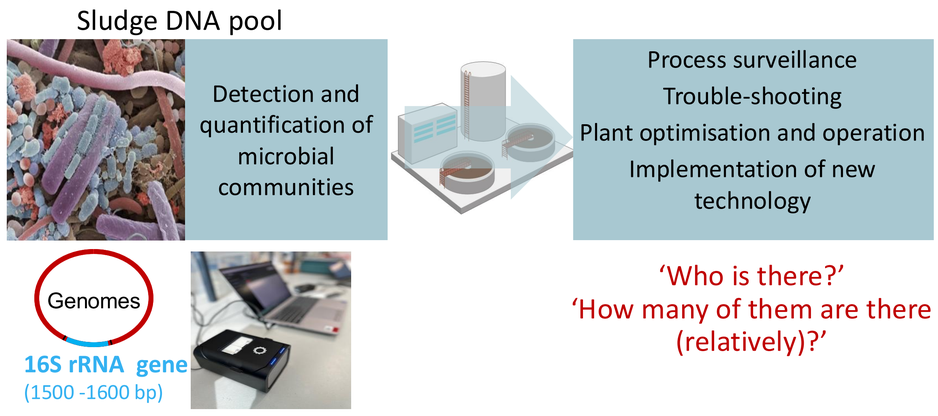


Figure 1. Sampling locations of Australian anaerobic digesters in 2020 for a global sequencing effort organized by Aalborg University (see Nature Communications by Dueholm et al. (2024) and coordinated by Damien Batstone (UQ). This project will do something similar but with a focus on Australia and with weekly samples over 1 full year.

Short read amplicon sequencing

Amplicon libraries (V3-V4 region, 16SrRNA as per Krohn et al. 2024) are sequenced on a PromethION2 Solo using Oxford Nanopore's Native Barcoding Kit and basecalled live using Dorado in super high-accuracy mode. Reads are quality-filtered, mapped, and classified against the MiDAS v5.3 reference database using a bespoke workflow that produces taxonomic abundance tables (Andersen et al., 2023).



Pilot Results

We propose to set up a national sludge sampling and DNA sequencing regime, where operators will access weekly taxonomic profiles from anaerobic digesters and aeration tanks and thus get seasonal and spatial data from thousands of process-relevant bacteria and archaea for the first time.

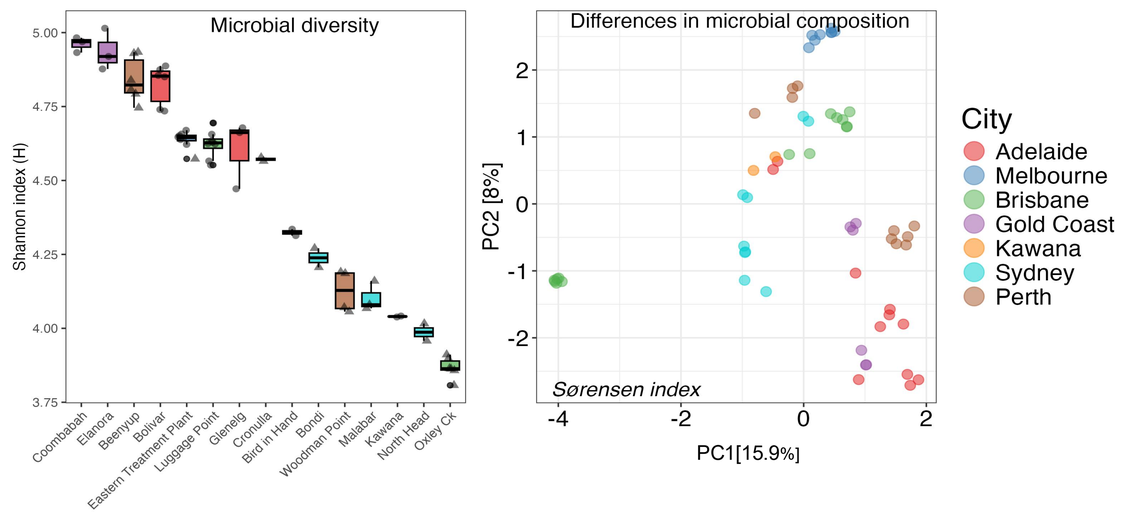


Figure 2. One outcome from this work will be the ability to benchmark the microbial diversity in their own reactors against national patterns to identify strengths, detect emerging risks, and guide evidence-based process optimization. LEFT: Comparison of total microbial diversity in Australian anaerobic digesters in January 2020. RIGHT: Differences in microbial composition between the same digesters.

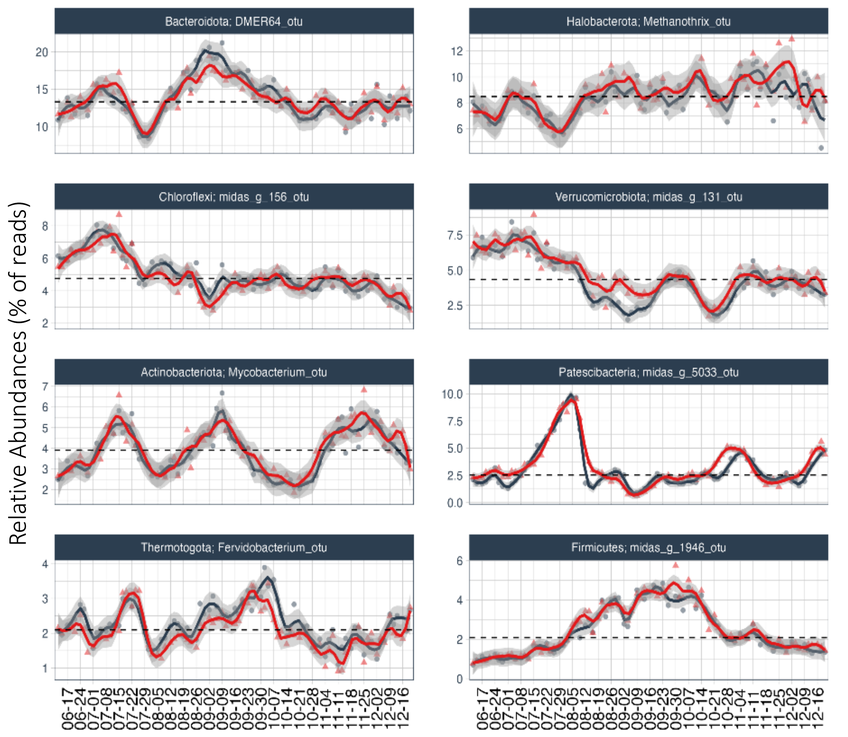


Figure 3. Example of relative abundances of some of the microbes in Australian anaerobic digesters over time. This data enables us to work towards a new decision support tool, informed directly from the biomass.

Outcomes for industry

Immediate	Short-medium term	Long-term
Knowledge transfer		
Number and type of microbes in your digesters. Understanding of biomass complexity and sensitivity. Understanding links to foaming risk.	Sector-wide DNA monitoring capabilities Marker gene sequencing workflow. High specificity profiling method. Onsite sequencing capability. Recruitment of new cohort of bioengineering graduates or experts. Discovery – linking microbiome and performance.	Software: Early warning systems DNA-to-screen monitoring tool. Operator-specific machine learning predictions. CH_4 capture, N_2O emissions, N removal, P removal, Pathogen detection.	Resource recovery enhancements Engineered consortia Tailored amendments Foam prevention Value-added products

References

Andersen, et al. "Fast DNA-Analyses for Surveillance of Microbial Communities in Full-Scale Deammonification Tanks: Potential for Control and Troubleshooting." Water Research 236 (2023): 119919.

Krohn, et al. "Dead in the Water – Role of Relic DNA and Primer Choice for Targeted Sequencing Surveys of Anaerobic Sewage Sludge Intended for Biological Monitoring." Water Research 253 (April 2024): 121354.

Dueholm, et al. "MiDAS 5: Global Diversity of Bacteria and Archaea in Anaerobic Digesters." Nature Communications 15, no. 1 (2024): 5361.