Using Metagenomics to Improve Wastewater Treatment

Sam Sarrafi, Rowan French

Priority Topic Area: **Water and Sanitation**

1 – Making Microbes Matter

Wastewater treatment relies on bacteria in secondary treatment to decompose organic pollutants. There is minimal understanding within the water industry of the "Biological Infrastructure" that drives this process.

On the behalf of United Utilities (UU) we are working in collaboration with Newcastle University, to explore Metagenomics as a pioneering solution. By sequencing microbial DNA, we can accurately identify the makeup of bacteria in wastewater treatment works. This innovation would allow operators to monitor microbial health, improve plant performance, provide early warning signs and help us to understand process emissions (specifically N_2O), which are accelerating climate change.

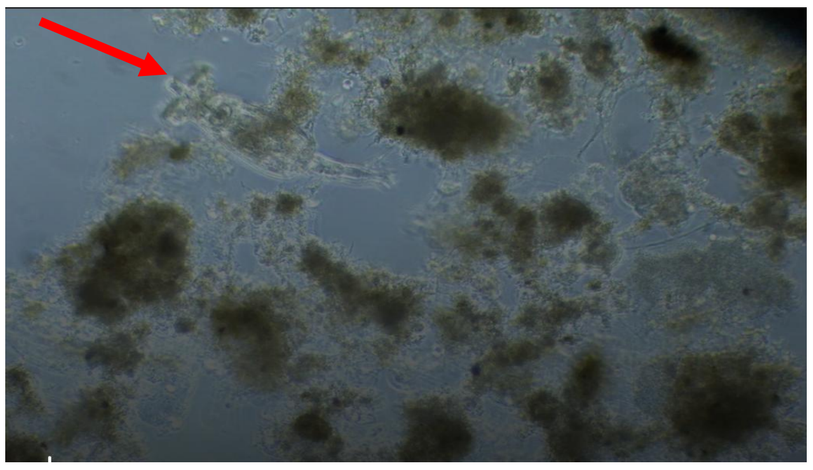


Figure 1: Activated sludge under the microscope (Rotifer highlighted).

Figure 2: Taking a sample for the Metagenomics trial.

2 – From Sample to Sequence

We took samples of activated sludge and froze them before sending them to Newcastle University for analysis. The analysis techniques include:

- **qPCR** - provides absolute abundance of specific organisms accurately by amplifying and counting DNA sequences.
- **16S** - estimates relative abundance based on a known DNA sequence but is not as accurate as qPCR.
- **Metagenomics** – characterises everything within a sample and bioinformatics is used to extract usable data

Our work has been split into two phases:

Phase 1 – We took singular samples from 10 wastewater treatment works (WwTWs) to identify sites of specific interest and to identify the relationships between sludge age, compliance and Ammonia Oxidising Bacteria (AOB) populations.

Phase 2 – We have taken weekly samples from 4 WwTWs to understand how bacteria populations change with seasons, change to sludge age, and to understand if changes in final effluent quality could have been predicted by bacteria populations.

Phase 2 is still in progress, with further analysis is expected Q2 2025.

3 – What's in the Water?

Initial results seen & potential future impact:

- We have developed a much greater understanding of how samples should be taken, stored and transported, enabling streamlining of future work.
- Foaming causing bacteria can be seen in samples before the event occurs (Figures 3 & 4). How this early warning sign can be used is being investigated further to understand how this would help operations make the best decisions.
- There is some evidence of a relationship between AOB biomass levels and compliance. Further analysis at a site level is being carried out to validate this and investigate it's use for predicting compliance failures.

Further Analysis Aims:

- Increased understanding of final effluent quality and river health.
- Observe sludge composition across seasons (focus Feb-Apr) targeting species specific to N_2O pathways.
- Calculate AOB population. This can be correlated with sludge age to find new set points to control the activated sludge process with.
- Understand if bacteria levels can be used to predict compliance issues.

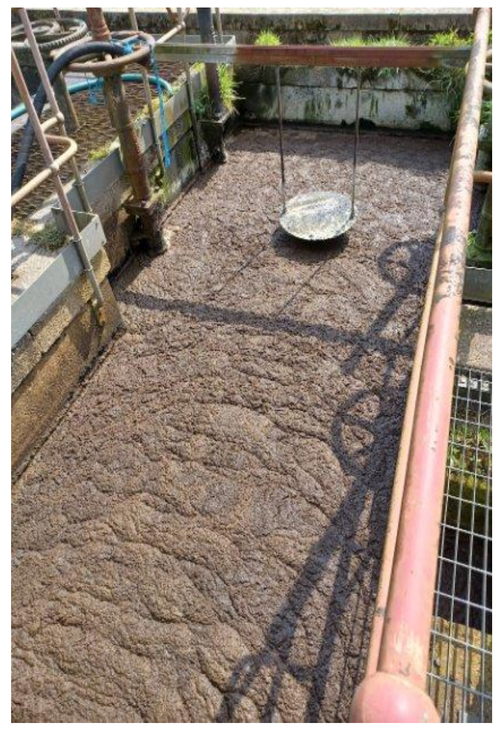
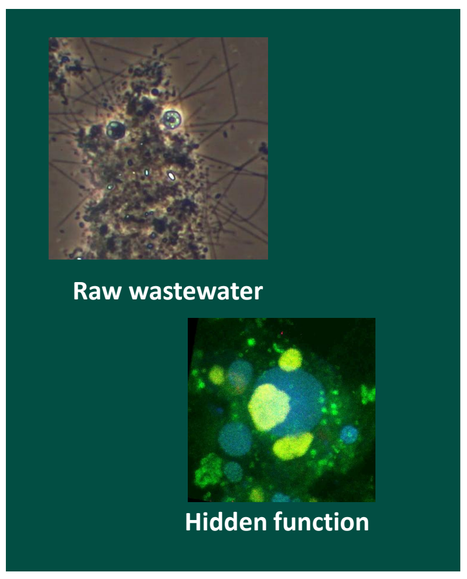
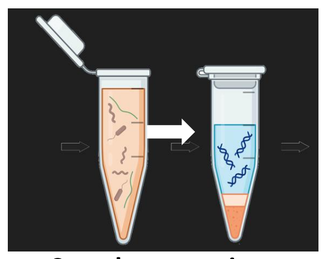


Figure 3: Foaming in an activated sludge process caused by unwanted bacteria.





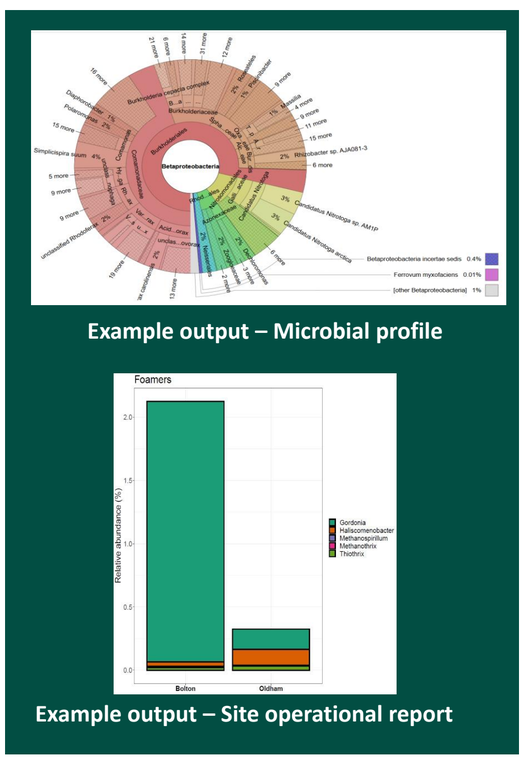


Figure 4: Metagenomics analysis overview.

4 – Driving Sustainability: Benefits of Metagenomics

Wastewater treatment is energy-intensive: **the water sector consumes around 2% of electricity in the UK.** Metagenomics will increase our understanding of microbial nitrification capacity, which could be used to drive energy efficiency.

Process emissions account for ~1/3 of the sector's estimated annual net emissions. Metagenomics will increase sector understanding of emissions pathways, enabling emissions reduction techniques to be tested and deployed. In turn, this will mitigate future carbon pricing risks and align with regulatory reduction incentives.

In the longer term, Metagenomics projects will support next generation wastewater management and digital solutions – optimising operability, cost, performance and greenhouse gas (GHG) emissions.

5 – Shaping the Future

An Ofwat bid for additional funding has been shortlisted for the Catalyst breakthrough judging. If successful, the project will explore the use of Metagenomics to assist wastewater operations and delve deeper into the production of process emissions.

References/Acknowledgements

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