KWR

DNA fingerprinting in surface- and wastewater management

Peer H.A. Timmers¹, Leo Heijnen¹, Joost van den Bulk², Susan Sollie², Edwin Kardinaal¹,Gertjan Medema^{1,3}

1 KWR watercycle institute, Groningenhaven 7, 3433 PE Nieuwegein, the Netherlands, **2** TAUW B.V., Australiëlaan 5, 3526 AB Utrecht, the Netherlands **3** Faculty of Civil Engineering and Geosciences, TU Delft, Stevinweg 1, 2628 CN Delft, the Netherlands

Background

In surface- and wastewater management it is important to determine the origin of the water and of the potentially problematic substances/organisms it might contain. These are faecal contamination, nutrient-related questions, or malfunctioning wastewater treatment plants (WWTPs). This research examines



KWR Watercycle Research Institute PO Box 1072, 3430 BB Nieuwegein, The Netherlands

Corresponding author peer.timmers@kwrwater.nl +31 (0)30 60 69 636



the possible contribution of the DNA fingerprinting technique High Throughput Sequencing (HTS) in clarifying such questions.



Challenges

Questions related to various water types were tried to be answered with HTS: 1. Is WWTP effluent discharge into surface water traceable? And if so, how far from the source?

Figure 2: Heatmap of HTS data of all WWTPs effluent and sewage samples. Shown are the average relative abundances (%) of the top 25 most abundant OTUs with a minimum abundance of 0.1% in all samples per location. OTUs were classified at the genus level.



Figure 3: Schematic representation of an example where we applied HTS as a tool to track changes overflow water and WWTP effluent discharge. It is expected that the water of stream 2 shows a different microbial profile before (sampling point 1) than after WWTP discharge (sampling point 3).

- Is the influence of sewer overflow into surface water, and are the different origins of the overflow, traceable?
- Is leakage of WWTP effluent into groundwater traceable? 3.
- 4. Is the origin of surface water that flows into other surface water traceable? These questions resulted in six research cases with Dutch water boards. One case is given as an example.

Results and discussion

- With HTS, we were able to distinguish sewage and WWTP effluent microbiomes (Figure 1).
- These microbiomes show characteristic OTUs for sewage and WWTP effluent (Figure 2).
- These microbiomes are sources that influence the microbial community on receiving waters. In our example case (Figure 3), the influence of these sources was traceable on the different sample locations and quantified using Source Tracker (Knight et al., 2011) (Figure 4).

Figure 4: Source tracker analysis of the relative amount (%) of influence of sewage or WWTP effluent on the different sampling locations (sample numbers from Fig 3). Samples were taken on different dates.



Figure 1: Principal Component Analysis of HTS data of all sewage and WWTP effluent samples from different sampling dates and different locations.

Conclusion

HTS is a powerful technique that can distinguish different sources that have a specific microbial fingerprint. These fingerprints can be detected specifically and with high sensitivity in sinks. This gives the opportunity to apply HTS to answer complex questions related to water management, where it is important to know the origin of different water bodies.

Acknowledgements

This project is a collaborative effort within the Topconsortium for Knowledge and Innovation Watertechnology (TKI). Participants include the Watercycle Institute KWR, TAUW consultancy, BaseClear and Water Boards and municipalities: Gemeente Utrecht, Hoogheemraadschap Hollands Noorderkwartier, Hoogheemraadschap van Rijnland, Hoogheemraadschap Stichtse Rijnlanden, Wetterskip Fryslan, Waterschap Hunze & Aa's, Waterschap Rivierenland, Waterschap Vallei & Veluwe.

References

Knight, D., J. Kuczynski, E. S. Charlson, J. Zaneveld, M. C. Mozer, R. G. Collman, F. D. Bushman, R. Knight and S. T. Kelley. (2011). Bayesian community-wide culture-independent microbial source tracking. Nat Methods, 8: 761.