Rapid microbial surveillance using nanopore DNA sequencing

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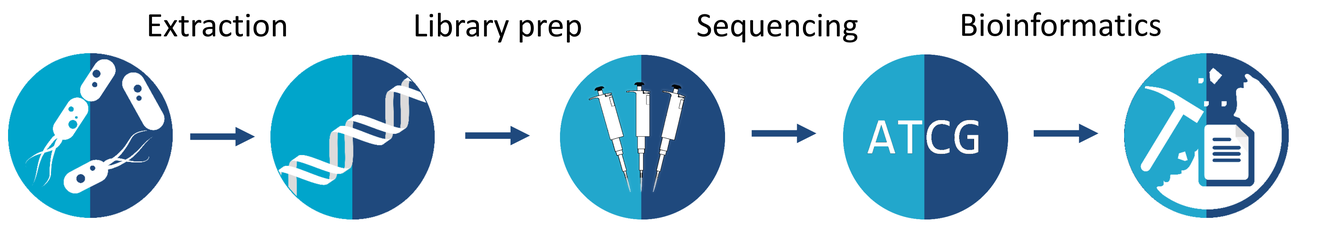
Introduction

Wastewater treatment is a key technology for both sanitation and resource recovery. The biological processes are generally stable, but occasionally problems occur. Certain bacteria are responsible for e.g. foaming, and should be kept at a low abundance. Golden standard DNA sequencing technology is not suitable for monitoring as the workflow is slow and unpractical. The MinION DNA sequencer from Oxford Nanopore Technology has enabled real-time sequencing and could potentially be applied routinely at wastewater treatment plants. This would give operators insights that could prevent process-critical problems.

Conclusions

- On-site DNA extraction worked and produced DNA for downstream application
- Library preparation was successful using miniPCR – could be faster
- The applied workflow could successfully obtain a community profile in 5.7 hours
- 30 minutes of sequencing was enough to create a stable community profile
- Bioinformatics limited by data transfer times. Local base-calling is a must

Methods



Extraction

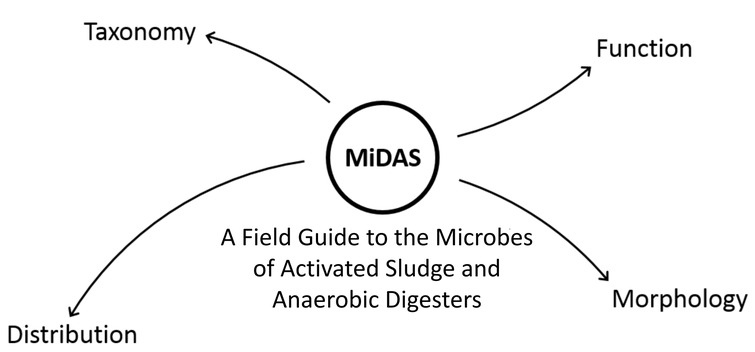
- Custom extraction method using some buffers from the FastDNA SPIN kit for Soil (MT BIO)
- Lysis using bead-beater with 3D printed adapter
- Purification using AMPure XP beads diluted 1:10
- Portable equipment: custom-made bead-beater, microfuge, Qubit fluorometer, pipettes and tips

Library & sequencing

- Full length 16S libraries created using Oxford Nanopore Rapid 16S amplicon barcoding kit
- Amplification performed on miniPCR thermal cycler using SQK-RAB201 protocol (30 cycles)
- Amplicon libraries sequenced on a MinION (Oxford Nanopore Tech.) with a v. 9.4 flow cell

Analysis

- Local base-calling (albacore), the resulting fastq files transferred to remote server for read mapping (minimap2) and file compression
- Taxonomy and functional categories assigned through MiDAS framework (local computer)
- Analysis of processed files using Rstudio through the ampvis2 package and the tidyverse

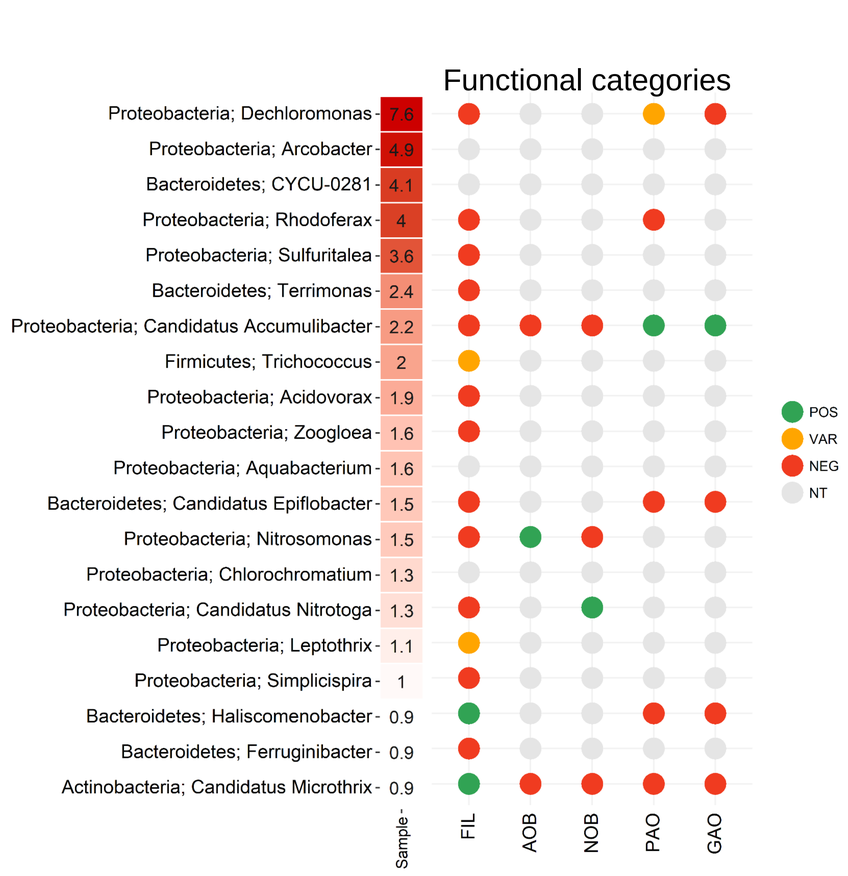


www.midasfieldguide.org

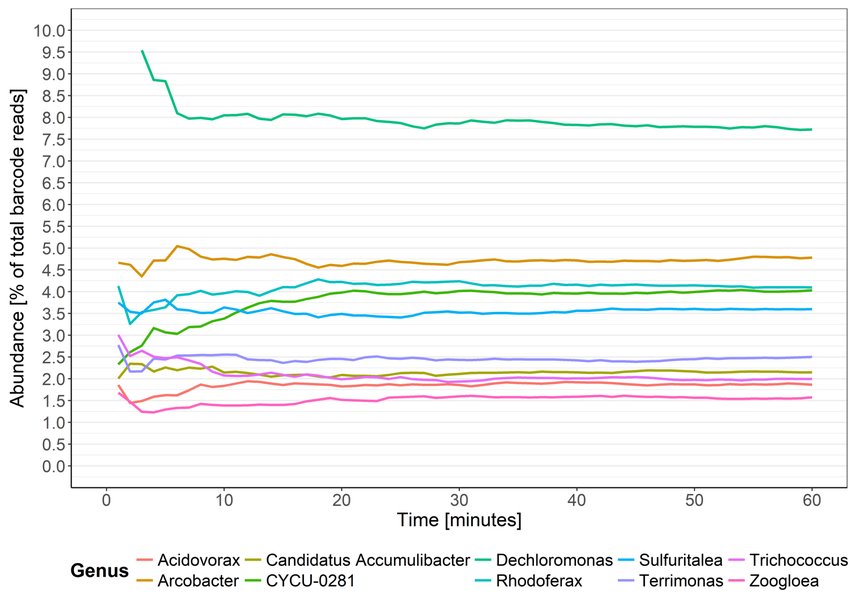
Future

- Potential for faster and better DNA extraction
- Sequencing metagenomes will make library preparation faster, VolTRAX could simplify prep
- Local base-calling will be possible real time, speeding up data processing and transfer times

Results



Heatmap of the 20 most abundant bacterial genera and their associated phyla, based on read abundance. Read abundance is shown in percent of total reads in the specific barcode. The sample was freshly sampled activated sludge, extracted and sequenced on-site. Functions of the genera were assigned through the MiDAS framework.



Read abundance of the top 10 most abundant genera over sequencing time. The abundance profile of most genera stabilises within 20 minutes, while others show a small degree of instability until 30 minutes of sequencing.

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