**Title: Managing the wastewater microbiome: Rapid microbial surveillance using Nanopore DNA sequencing**

**Martin Hjorth Andersen1, Rasmus Hansen Kirkegaard1, Mads Albertsen1**1 Center for Microbial Communities, Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, 9220 Aalborg Ø, Denmark  
[mha@bio.aau.dk](mailto:mha@bio.aau.dk)

**Background**

Wastewater treatment plants depend heavily on microbial communities to clean sewage water, which has to pass strict nutrient requirements before the effluent goes into waterways. The biological processes are generally stable. However, problems do occur occasionally, can arise quickly and lead to process breakdown, leading to increased operational costs and potential environmental hazards[1,2]. With current methods, it is often impossible to predict a system crash before it is too late. Monitoring the microbial community for critical changes is tedious, as the process from sample to results generally take several days and requires expert knowledge as well as expensive lab facilities. A faster workflow is therefore necessary to ensure a more efficient wastewater management in the future.

**Experimental design**

Protocols were developed with focus on portability, ease-of-use and equipment cost. DNA extraction was performed with a mobile bead beater with a 3D-printed adapter, a table centrifuge and beads for solid phase reversible immobilization (SRPI beads). Full-length 16S rRNA amplicon libraries were produced using a miniPCR™ (Amplyus, USA) and the SQK-RAB201 kit from Oxford Nanopore (Oxford Nanopore Technologies, UK). The amplicon libraries were sequenced on a MinION (Oxford Nanopore Technologies, UK) and data for the first 10 minutes of sequencing was base-called on a local laptop, mapped to the MiDAS database using minimap2 and analysed in the R environment using the R package ampvis2[3].

**Results and Discussion**

The developed workflow enabled us to go on-site a wastewater treatment plant and perform the workflow from sampling to a resulting community composition profile in 6 hours. This rapid workflow could currently provide actionable information to plant operators in time to mitigate a process breakdown, and based on ongoing developments it is our belief that plant operators will soon be able to monitor and report the microbial status as a routine measurement alongside simple process characteristics such as pH and temperature. With further improvements the workflow will be simple enough for on-site lab technicians, without experience in molecular biology, to perform the complete workflow per routine.

**Literature**

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