

# Global Perspectives on Activated Sludge Community Composition analyzed using 16S rRNA amplicon sequencing

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**Abstract:** Activated sludge is the most commonly applied bioprocess throughout the world for wastewater treatment. Microorganisms are key to the process, yet our knowledge of their identity and function is still limited. High-throughput 16S rRNA amplicon sequencing can reliably characterize microbial communities, and in this study activated sludge sampled from 32 Wastewater Treatment Plants (WWTPs) around the world was described and compared. The top abundant bacteria in the global activated sludge ecosystem were found and the core population shared by multiple samples was investigated. The results provide novel insights into the global diversity of activated sludge community and provide a baseline to study the factors driving bacterial assembly in WWTPs and prioritize further work aiming at understanding their ecological function.

Keywords: Activated sludge, Amplicon sequencing, Wastewater treatment

## Introduction

The activated sludge process is successfully applied worldwide for wastewater treatment. Information about the composition of activated sludge microbes and their function is essential for process performance and optimization, however, recently celebrating 100-years of activated sludge, our knowledge thereof is still somewhat limited (Nielsen and McMahon, 2014). DNA based identification of microorganisms using 16S rRNA amplicon sequencing has been developed over the past few years and is now ready to use for comprehensive studies of community profiling thanks to short analysis time, low cost, high throughput, and high taxonomic resolution. In this study we investigated microbial community composition of 32 globally distributed full-scale Enhanced Biological Phosphorus Removal (EBPR) Wastewater Treatment Plants (WWTPs) from twelve countries using 16S rRNA amplicon sequencing technology and a curated MiDAS taxonomy (McIlroy et al., 2015). The data presented here gives the first comprehensive insight into the microbial community composition of activated sludge across the world.

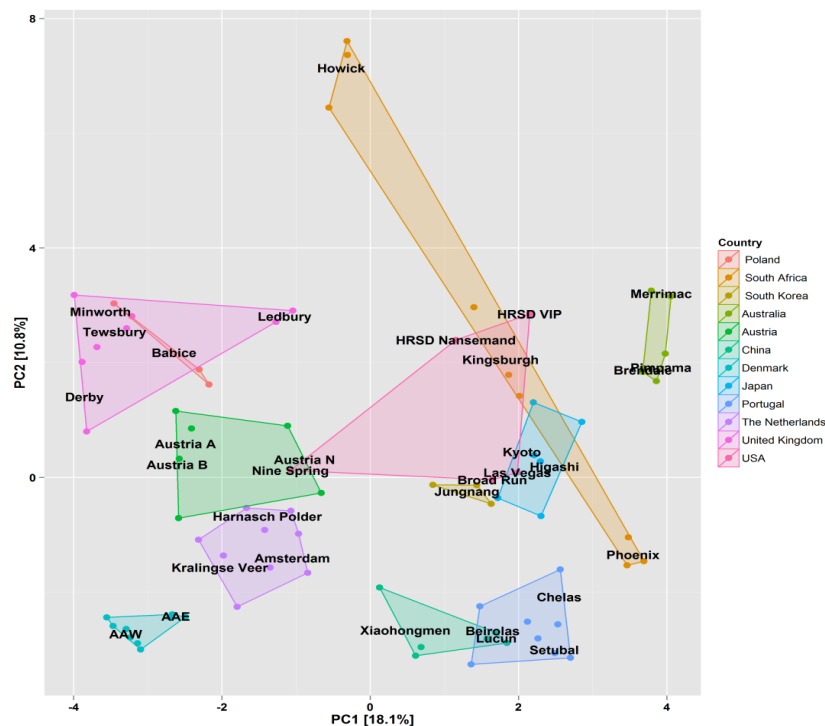
## Materials and Methods

Activated sludge samples were collected from the aeration tanks of 32 WWTPs with EBPR configuration located worldwide. 10 ml sample in triplicates were freeze-dried and shipped to Aalborg University, Denmark. DNA was extracted, samples were prepared (using primers targeting V1-3 region of 16S rRNA gene) and sequenced using protocols and workflows published by MiDAS webpage <http://www.midasfieldguide.org/en/protocols/>. Amplicon bioinformatic processing was performed as described in Albertsen *et al.* (2015) using curated MiDAS taxonomy

for classification (McIlroy *et al.*, 2015). The results were visualized in R (R Core Team, 2015) through R studio interface (<http://www.rstudio.com>) and analyzed using ampvis package (Albertsen *et al.*, 2015).

## Results and Conclusions

The microbial community composition of 32 nutrient removal WWTPs from across the world was successfully characterized by 16S rRNA amplicon sequencing. Similarities of activated sludge organisms were explored and the top abundant bacteria were elucidated.

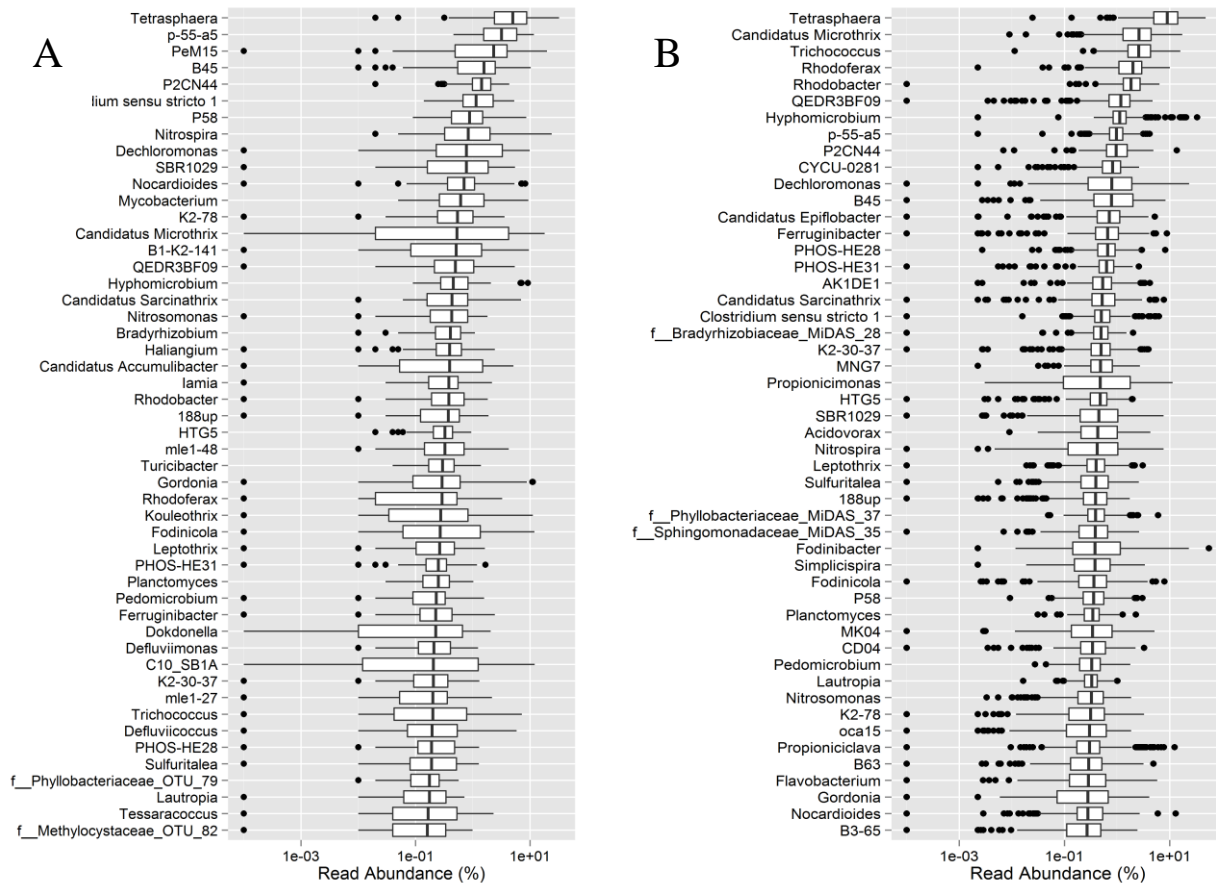


**Figure 1.** PCA analysis of microbial community of 32 EBPR WWTPs located in 12 countries across the world.

The similarity of the samples was evaluated using Principal Component Analysis (Fig. 1). Samples from WWTPs located in the same country grouped closely together, as did the countries located in the same geographical region indicating that the temperature and climate can be an important factor driving the difference in microbial community structure. Still, the influence of other abiotic factors that shape the community diversity composition remains to be investigated.

Based on the relative abundance results obtained by amplicon sequencing, the average abundance for each of the identified bacteria was calculated for all the samples analyzed, and the list of top 50 abundant microorganisms in activated sludge of EBPR full-scale WWTPs is presented in Fig. 2A. The results were compared to the top 50 bacteria identified in the 8-years long survey of 24 Danish WWTPs (McIlroy *et al.*, 2015) (Fig. 2B). 62% of the top 50 bacterial genera were shared between those 2 surveys indicating that the same microorganisms are present in activated sludge systems around the globe although their relative abundance differs. The *Tetrasphaera* genus, identified as a

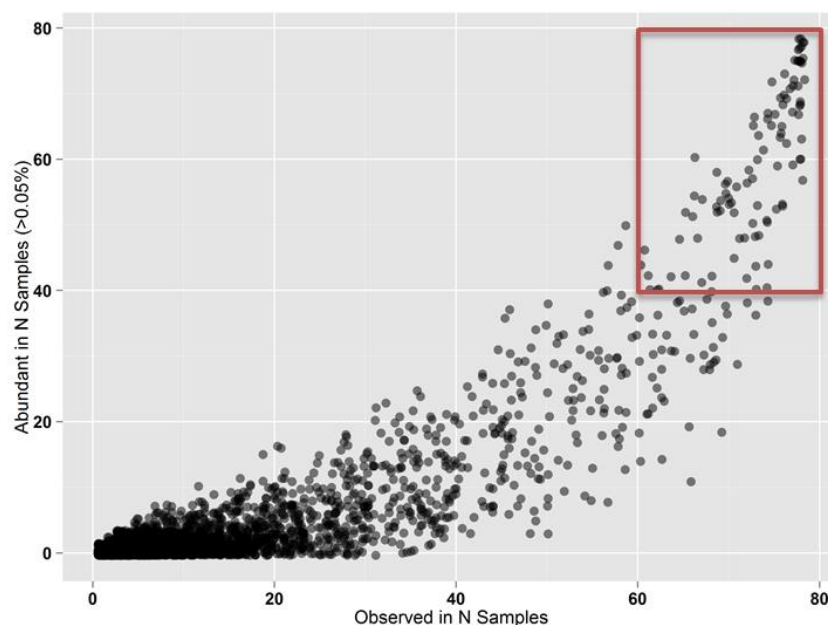
Polyphosphate Accumulating Organism, PAO (Nguyen *et al.*, 2011), was shown to be the most abundant bacteria in EBPR WWTPs in both surveys. Interestingly, a high fraction of bacteria found among these top 50 microorganisms is not functionally characterized and has presently only a number as ID (e.g. p-55-a5, PeM15 and B45 genera), thus their role in activated sludge process is unknown.



**Figure 2.** Top 50 most abundant bacteria (genus level) identified in global (A) and Danish (B) survey, sorted by average abundance in all the samples investigated.

Previous studies have suggested the existence of core community in activated sludge based on the analysis of number of Danish (Saunders *et al.*, 2015) and Chinese and North American (Zhang *et al.*, 2012) WWTPs. In this study, the core community was defined in the region marked in red in Fig. 3 denoting the bacteria (OTU level) that are both frequently present ( $\geq 77\%$  of samples) and frequently abundant ( $\geq 51\%$  of samples). All the analyzed plants contained a core population of 93 genus-level bacteria that made up 64% of the total bacteria present suggesting that the factors driving an assembly of activated sludge community in EBPR plants are similar across the globe.

Understanding microbial ecology requires knowledge about the community composition and function of the microorganisms present. Since functional characterization is not a trivial task (functional characterization of all microorganisms present is literally impossible), resource allocation should be well considered. This study elucidated the most abundant organisms present in



**Figure 3.** Frequency distribution of bacteria (OTUs) across all the samples analyzed. Data points represent individual OTUs.

This study elucidated the most abundant organisms present in activated sludge worldwide. For a high fraction of these no functional information is available, thus the obtained results can be used to prioritize further work aiming at understanding their ecological function and optimizing activated sludge process. As suggested previously (Saunders *et al.*, 2015) and confirmed by the results of this study, the knowledge of activated sludge ecology based on thorough investigations of limited number of plants can be of general importance for all treatment plants employing activated sludge process.

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