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High throughput 16S rRNA gene amplicon sequencing

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Nierychlo, Marta; Larsen, Poul; Jørgensen, Mads Koustrup; Albertsen, Mads; Karst, Søren Michael; Christensen, Morten Lykkegaard; Nielsen, Per Halkjær

Publication date:
2014

Document Version
Early version, also known as pre-print

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Nierychlo, M., Larsen, P., Jørgensen, M. K., Albertsen, M., Karst, S. M., Christensen, M. L., & Nielsen, P. H. (2014). *High throughput 16S rRNA gene amplicon sequencing: A fast and cheap method to study the influence of microbial community composition on activated sludge floc properties*. Poster presented at Activated sludge - 100 years and counting, Essen, Germany.

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High throughput 16S rRNA gene amplicon sequencing: a fast and cheap method to study the influence of microbial community composition on activated sludge properties



Marta Nierychlo, Poul Larsen, Mads K. Jørgensen, Mads Albertsen, Søren M. Karst, Morten L. Christensen, Per H. Nielsen

Center for Microbial Communities
Aalborg University, Aalborg, Denmark



mni@bio.aau.dk

Introduction

A reliable and reproducible method for identification and quantification of microorganisms is important for the studies of microbial communities in activated sludge and for the demonstration of their significance for plant operation and stability.

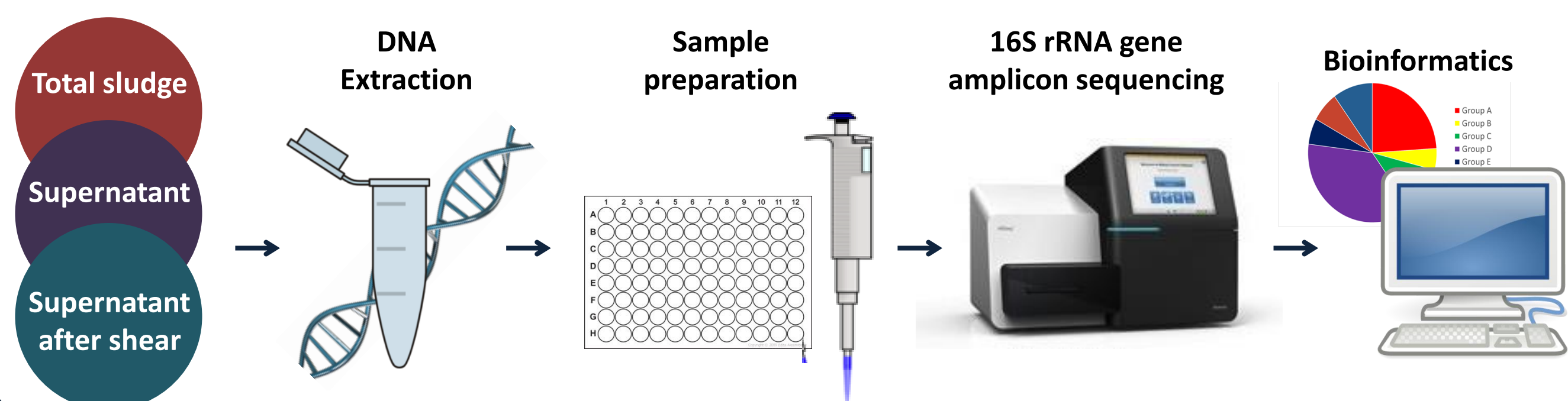
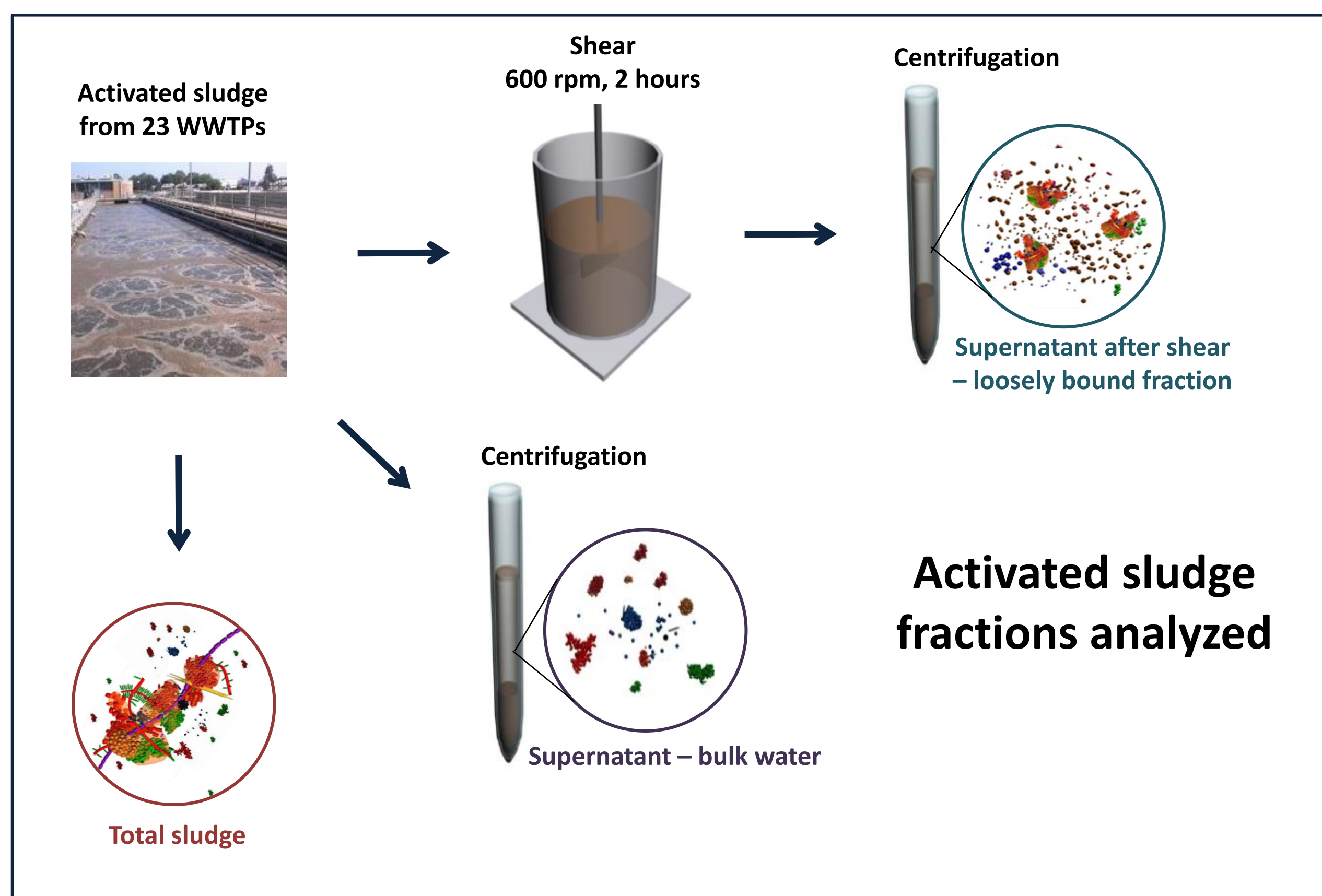
DNA-based identification of microorganisms using 16S rRNA gene amplicon sequencing has been developed over the past few years and is now ready to use for more comprehensive studies related to plant operation and optimization thanks to short analysis time, low cost, high throughput, and high taxonomic resolution.

Since bacterial morphology, mode of growth and EPS composition determine floc size, shape and strength, which in turn influence important sludge properties, the link established between the microbial community structure and physico-chemical sludge characteristics may provide a better understanding of the activated sludge process.

Objectives

- To show how 16S rRNA gene amplicon sequencing can be used to reveal factors of importance for the operation of 23 full-scale nutrient removal plants that can be related to settling problems and floc properties.
- To investigate whether the microbial community composition differs between the flocs and the supernatant (bulk water) and whether certain bacterial species are prone to detachment from the flocs.

Methods



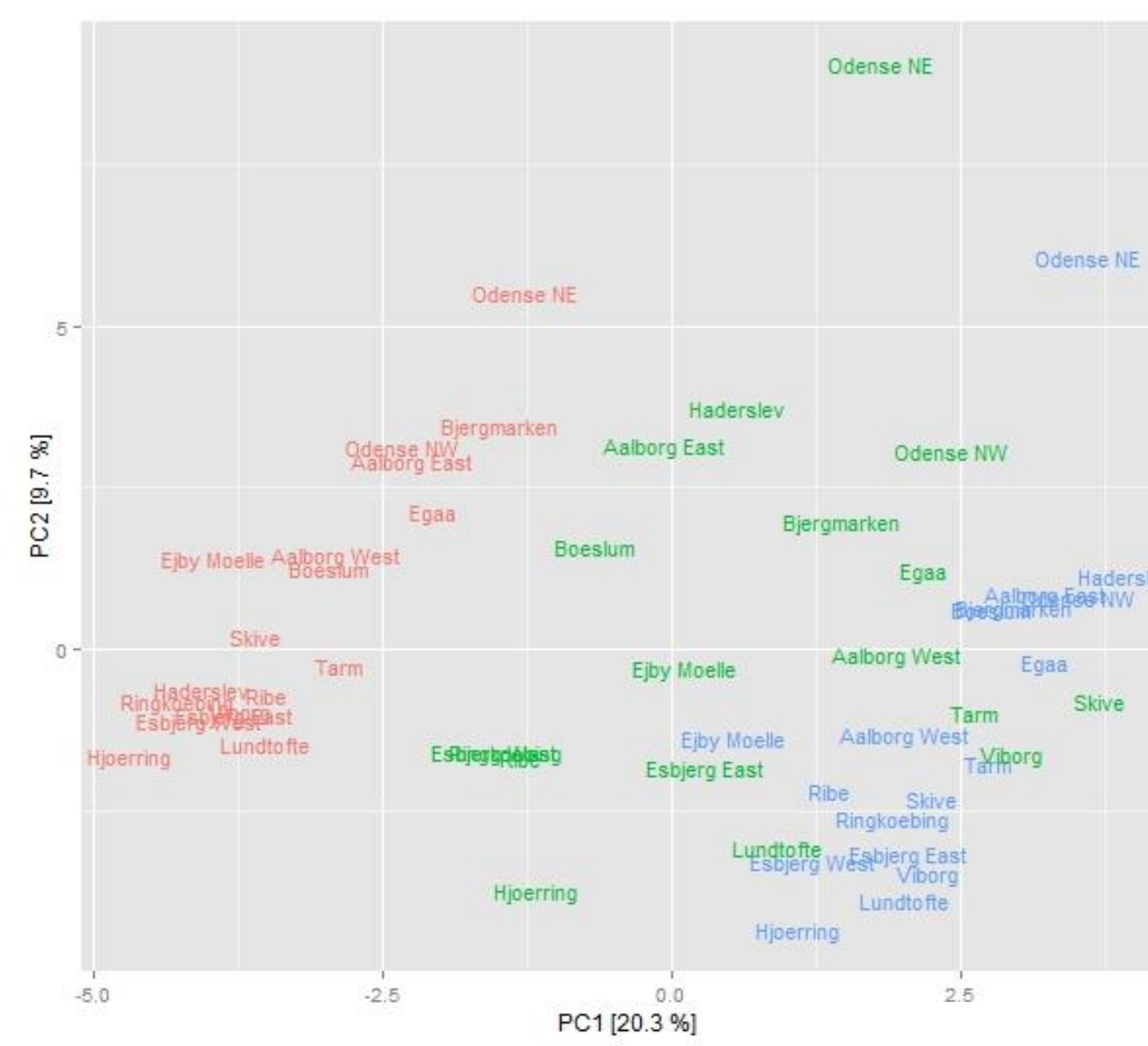
Conclusions

16S rRNA gene amplicon sequencing is suitable for comprehensive studies of WWTPs thanks to short analysis time, low cost, high throughput, and high taxonomic resolution.

A number of bacterial species can be correlated to the sludge characteristics that are important for the proper plant operation (SVI, floc strength, and EPS content).

Specific bacteria are enriched in the bulk water fraction and in the fraction loosely bound to the floc.

Results



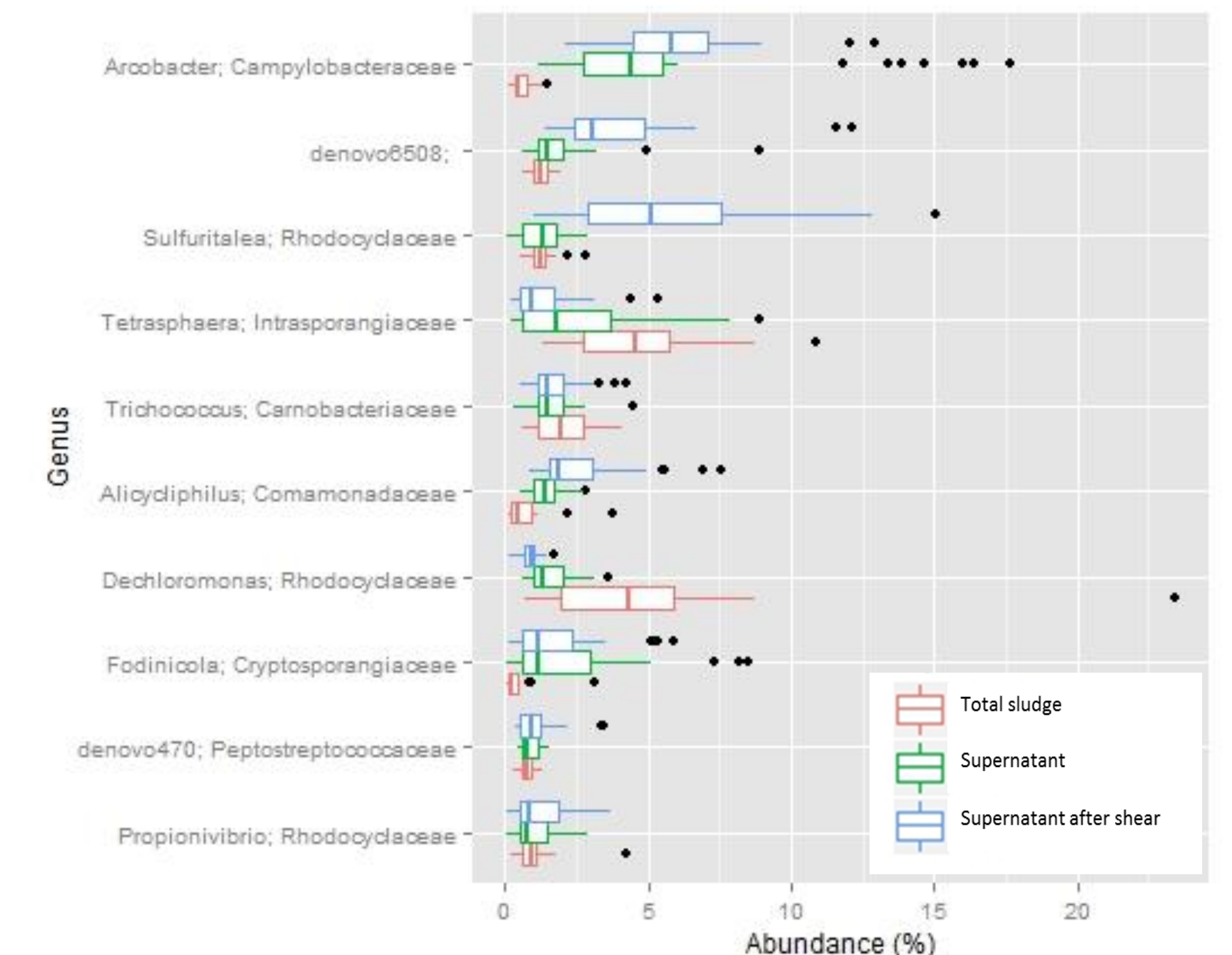
Community composition in different activated sludge fractions

The figure shows the relationship between all samples analyzed from 23 WWTPs.

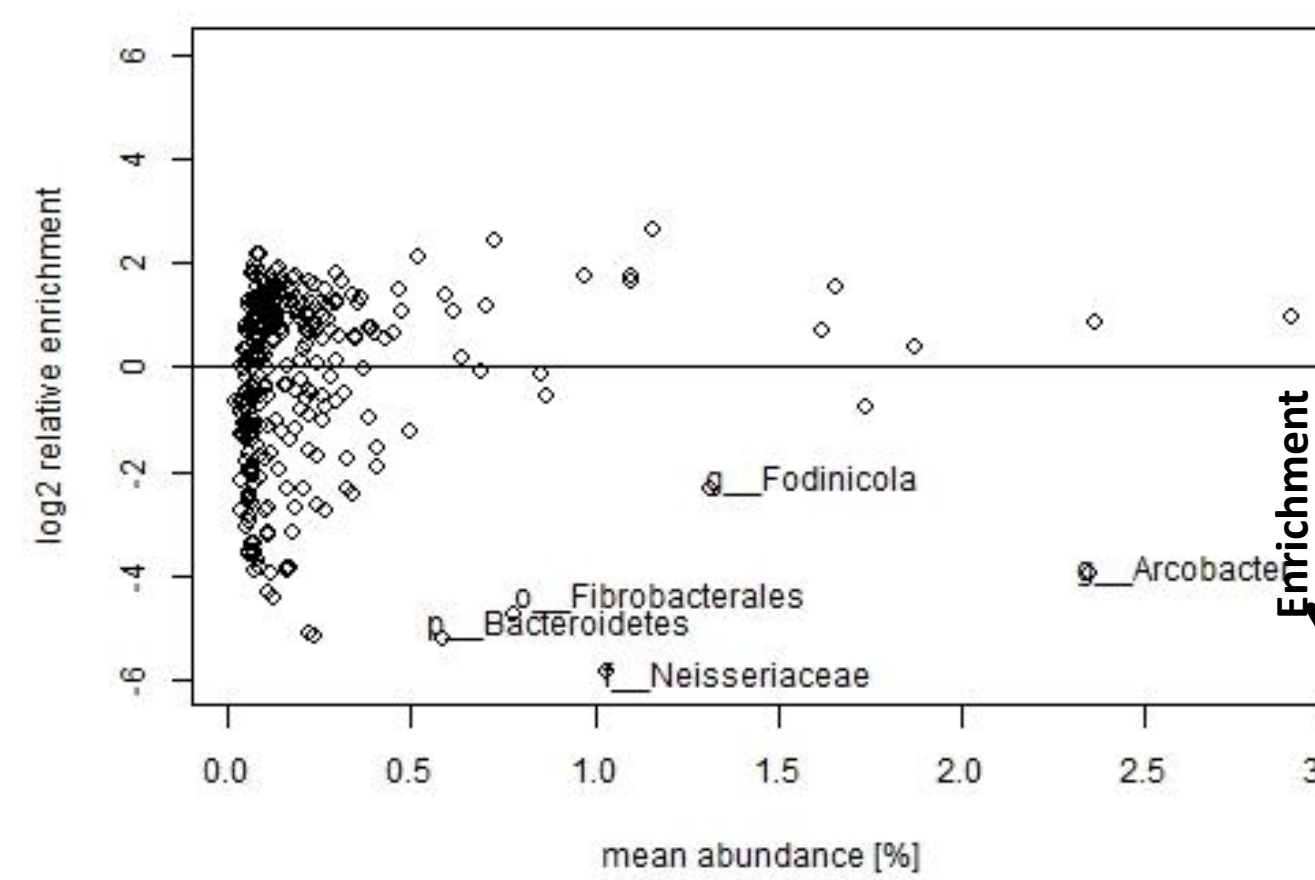
Samples grouping into individual sludge fractions (total sludge – bulk water – loosely bound fraction) can be clearly observed.

Relative abundance of 10 most frequently occurring genera compared between the different sludge fractions.

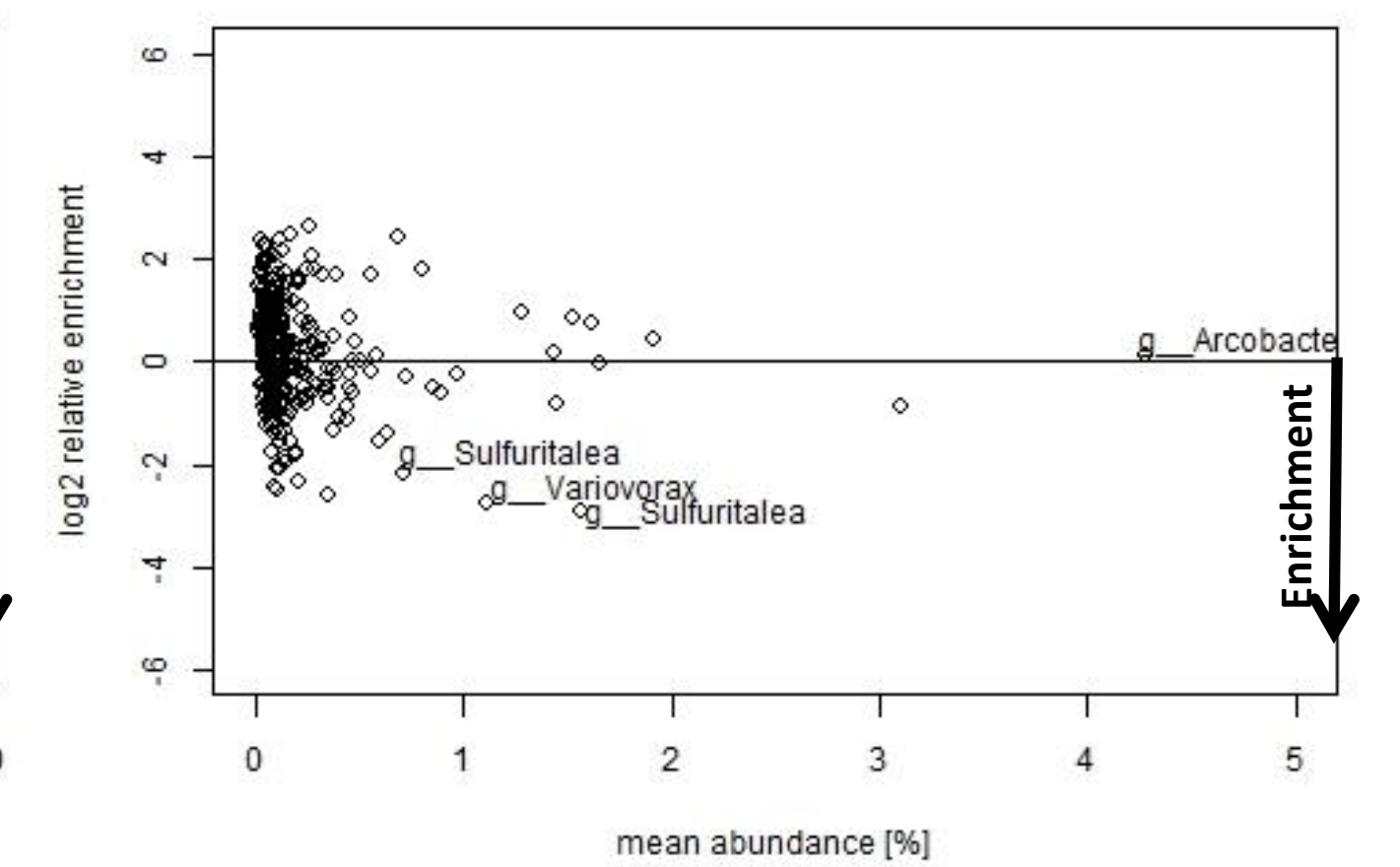
The variation between the WWTPs is captured by the boxplot width.



Bacteria enriched in the supernatant compared to total sludge



Bacteria enriched in the supernatant after shear compared to supernatant



SLUDGE VOLUME INDEX					
Total sludge		Supernatant		Supernatant after shear	
OTU	taxonomic classification	R	OTU	taxonomic classification	R
276	Lactococcus	0.8	276	Lactococcus	0.7
979	Arcobacter	0.8	632	Arcobacter	0.8
1188	Caldilineaceae	0.7	1188	Caldilineaceae	0.8
1188	Caldilinea	0.7	8679	Fodiniibacter	0.8
1230	Pseudorhodobacter	0.7	1232	Streptococcus	0.8
4117	Gordonia	0.7	10334	Thermomonas	0.7
8679	Fodiniibacter	0.7	203	Alicyclophilius	0.7
1232	Streptococcus	0.7	1232	Nocardioideae	0.6
5480	Nocardioideae	0.6	11578	Variovorax	0.7
564	Ferruginibacter	0.6	6976	Cloacibacterium	0.7
790	Chitinophagaceae	0.6	4241	SMA 35	0.6
6268	Leucobacter	0.6	6930	HF_BF 39	0.7
185	AB37	0.7	1929	Unclassified	0.7
40	R063	0.7			

SHEAR SENSITIVITY					
Total sludge		Supernatant		Supernatant after shear	
OTU	taxonomic classification	R	OTU	taxonomic classification	R
995	Trichococcus	0.6	5179	Sulfuritalea	0.6
4117	Chitinophagaceae	0.6	12320	Agromyces	0.6
203	Alicyclophilius	0.6	3387	Geothrix	0.6
1232	Pseudorhodobacter	0.6	1448	Lamia	0.6
1581	Propionivibrio	0.7	15576	Nitrosomonas	0.6
1043	Propionivibrio	0.7	6535	CFB38709	0.7
4117	Thermomonas	0.6	9919	Tha44	0.6
9919	Tha44	0.7	14300	Propionibacteriaceae	0.6
14300	Propionibacteriaceae	0.6	394	Hyphomicrobium	0.6
184	Hyphomicrobium	0.6	3235	Kinetosphaera	0.7
1012	Leucobacter	0.6	1232	Geothrix	0.6
203	Alicyclophilius	0.6	13177	WCHB140	0.6
601	Ferruginibacter	0.6	5410	Chloroflex	0.6
1232	Pseudorhodobacter	0.6	4763	Rhodococcus	0.6
388	Thermomonas	0.6	1051	Hyphomicrobium	0.6
			1051	Hyphomicrobium	0.6

Spearman correlation of bacteria present in different sludge fractions with important sludge characteristics: Sludge Volume Index (SVI), shear sensitivity, degree of flocculation and conditions for flocculation. Bacteria that were highly correlated ($R > 0.6$) with the mentioned parameters are listed above.