



Aalborg Universitet

AALBORG UNIVERSITY  
DENMARK

## 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*

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.

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- ? Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- ? You may not further distribute the material or use it for any profit-making activity or commercial gain
- ? You may freely distribute the URL identifying the publication in the public portal ?

### Take down policy

If you believe that this document breaches copyright please contact us at [vbn@aub.aau.dk](mailto:vbn@aub.aau.dk) providing details, and we will remove access to the work immediately and investigate your claim.

# 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.

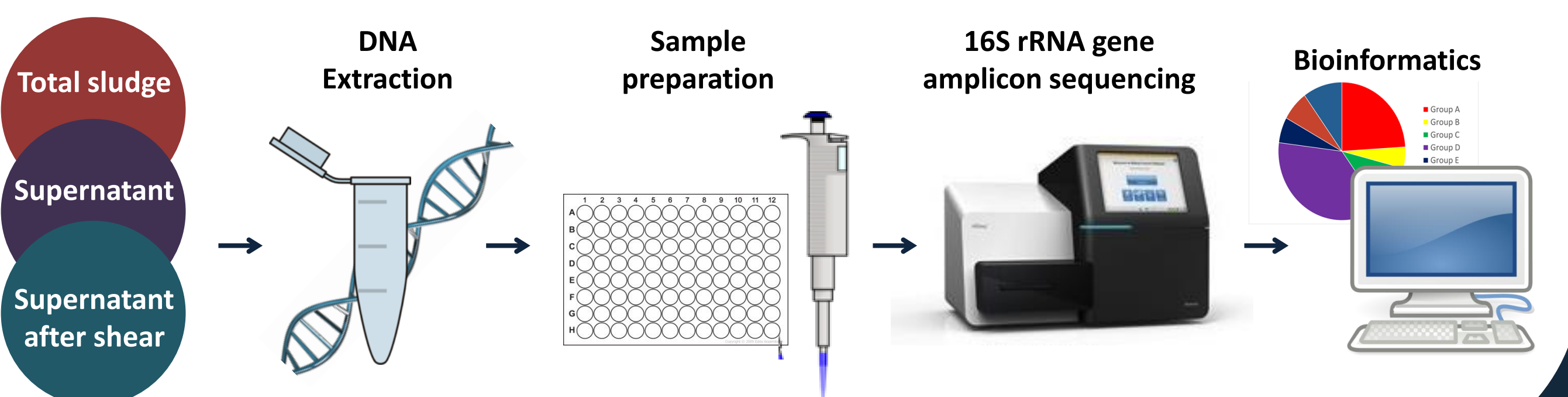
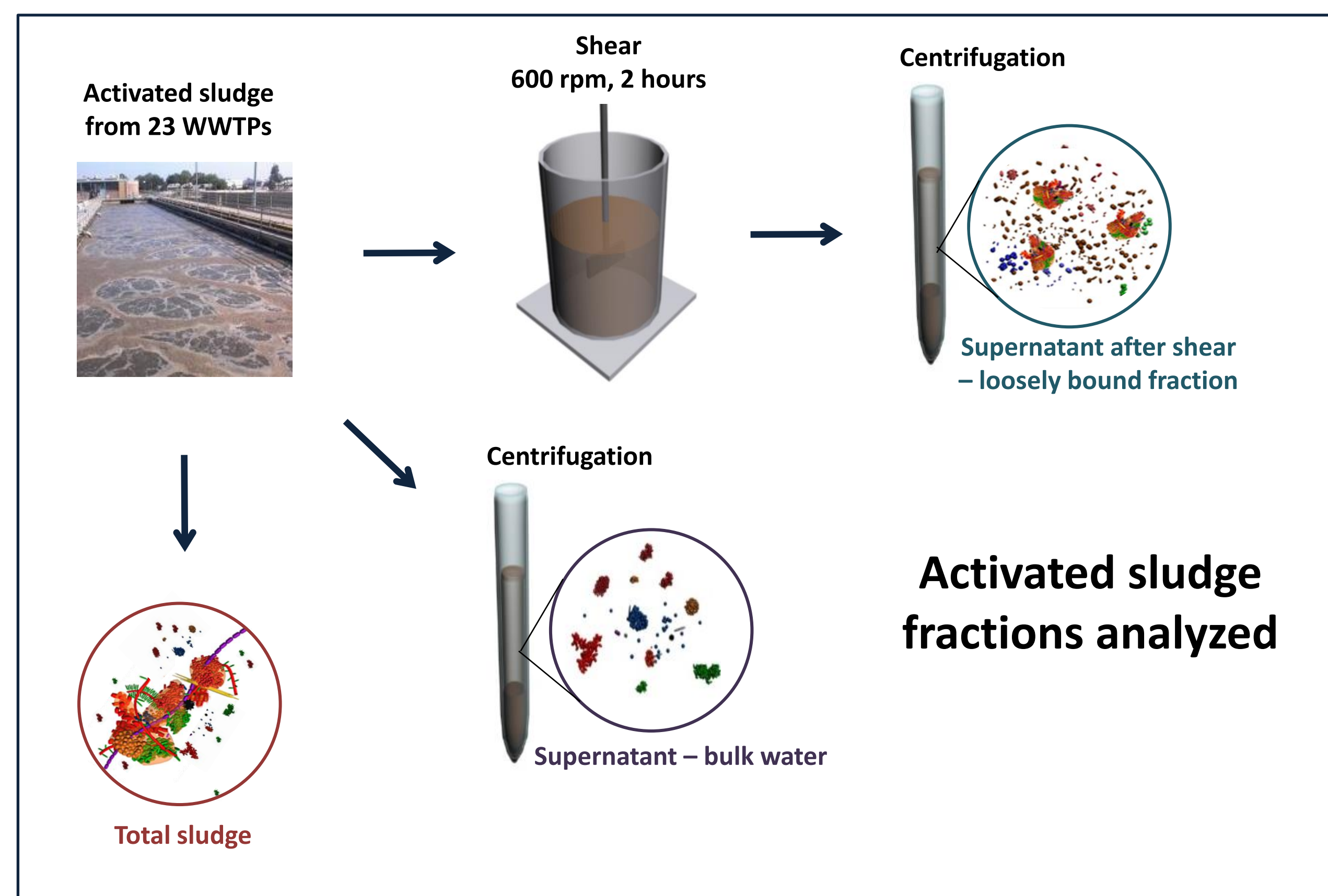
DNA-based identification of microorganisms using 16S rRNA gene amplicon sequencing has been developed over the past few years and is now ready to use for more comprehensive studies related to plant operation and optimization thanks to short analysis time, low cost, high throughput, and high taxonomic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



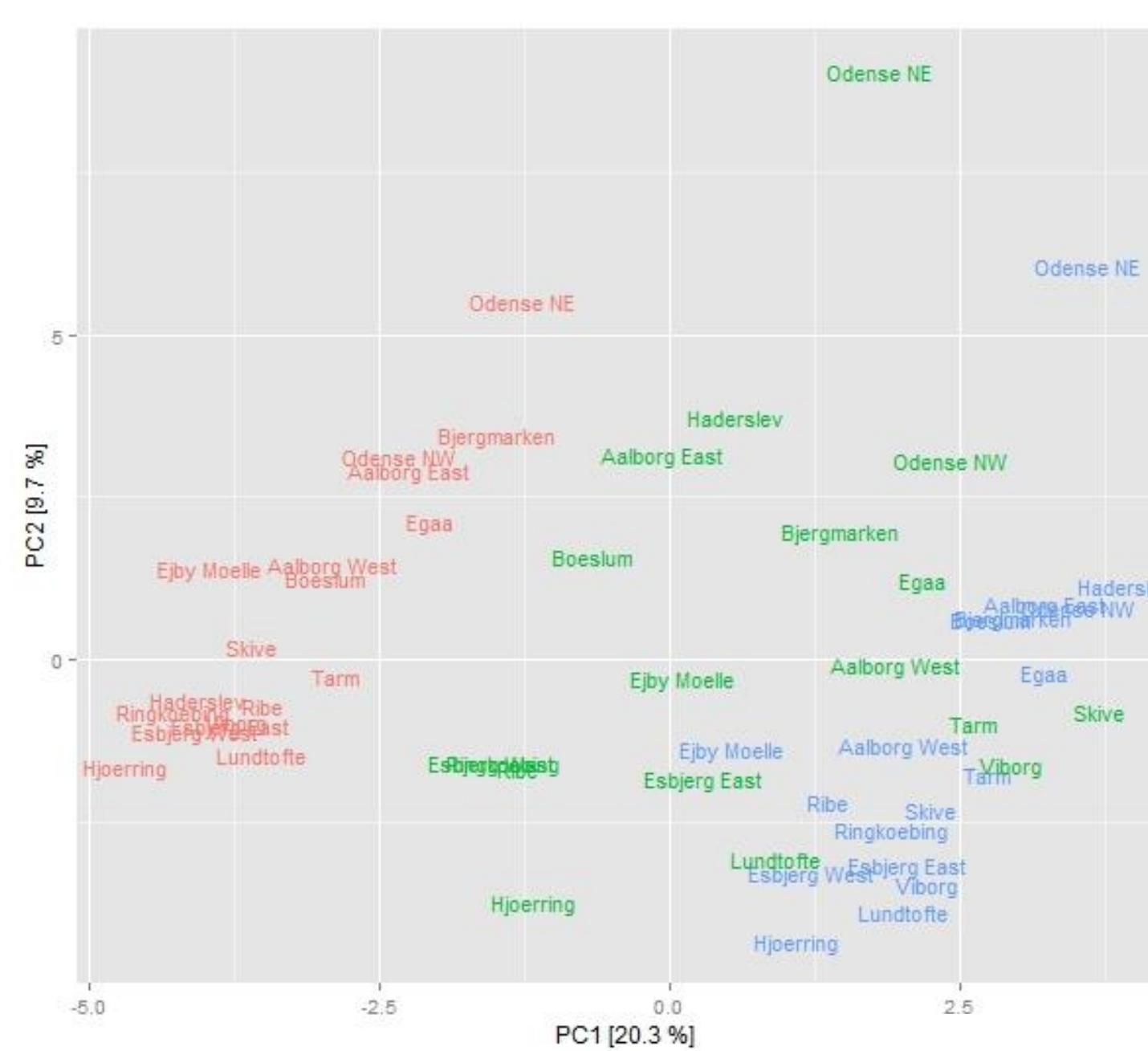
## Conclusions

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.

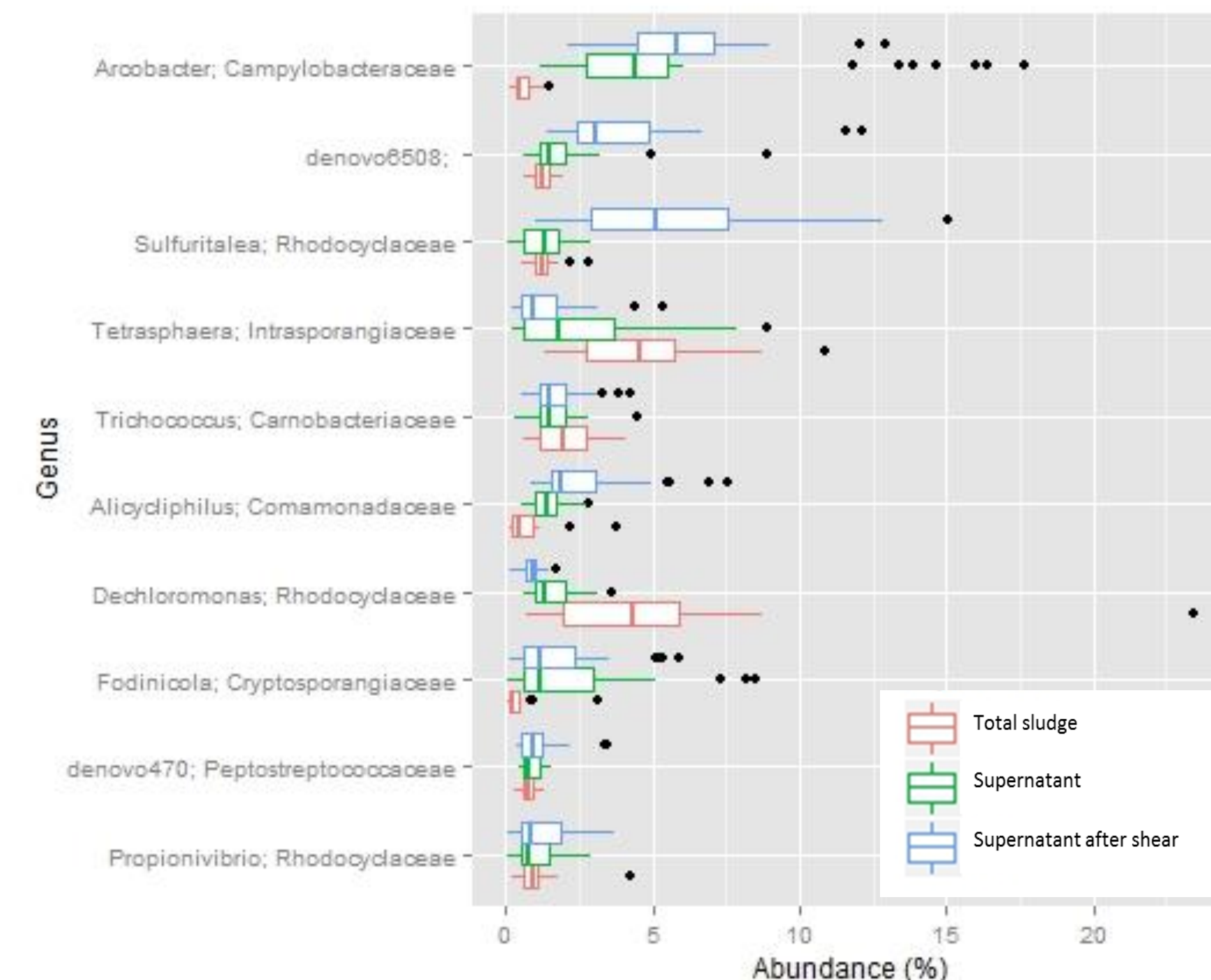
## Results



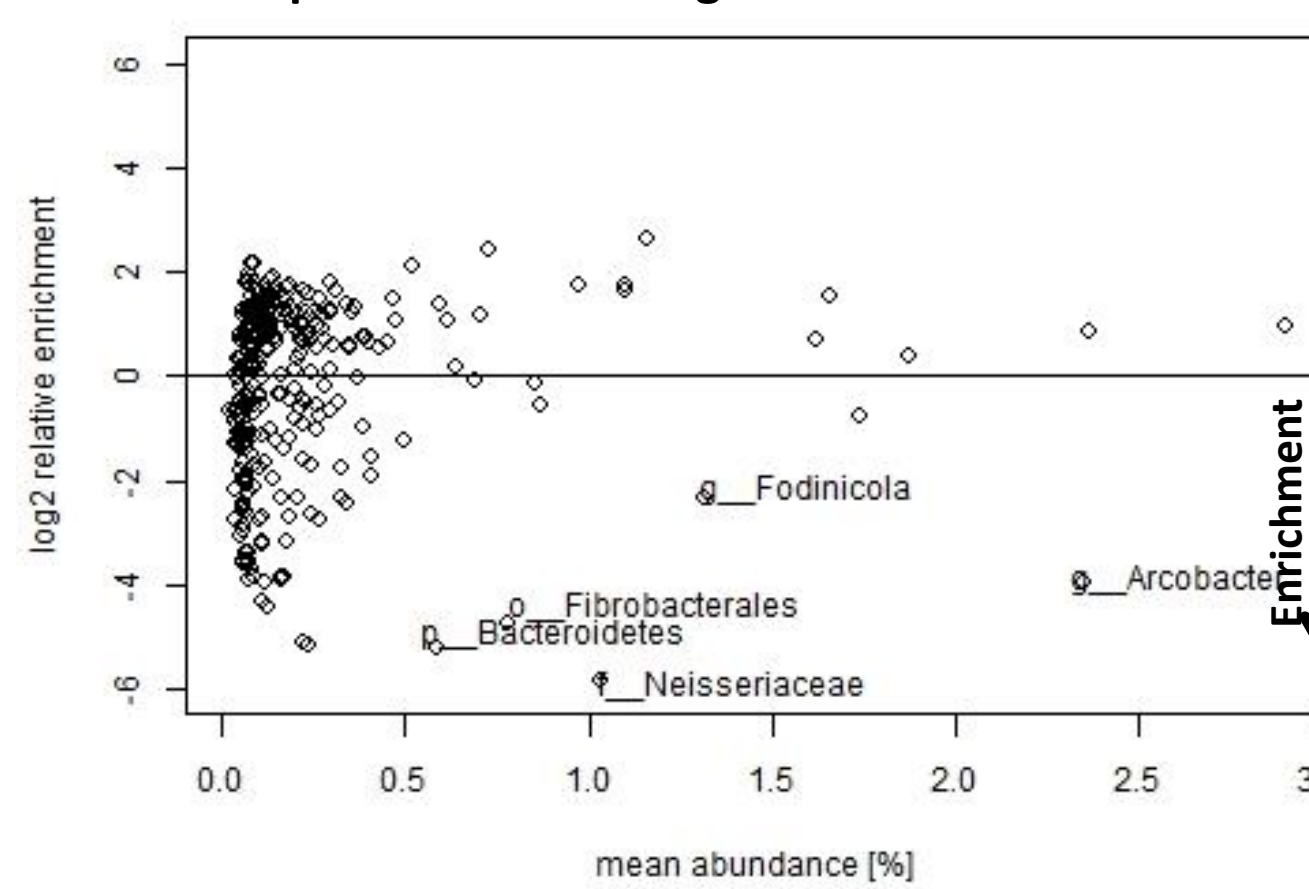
### Community composition in different activated sludge fractions

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.

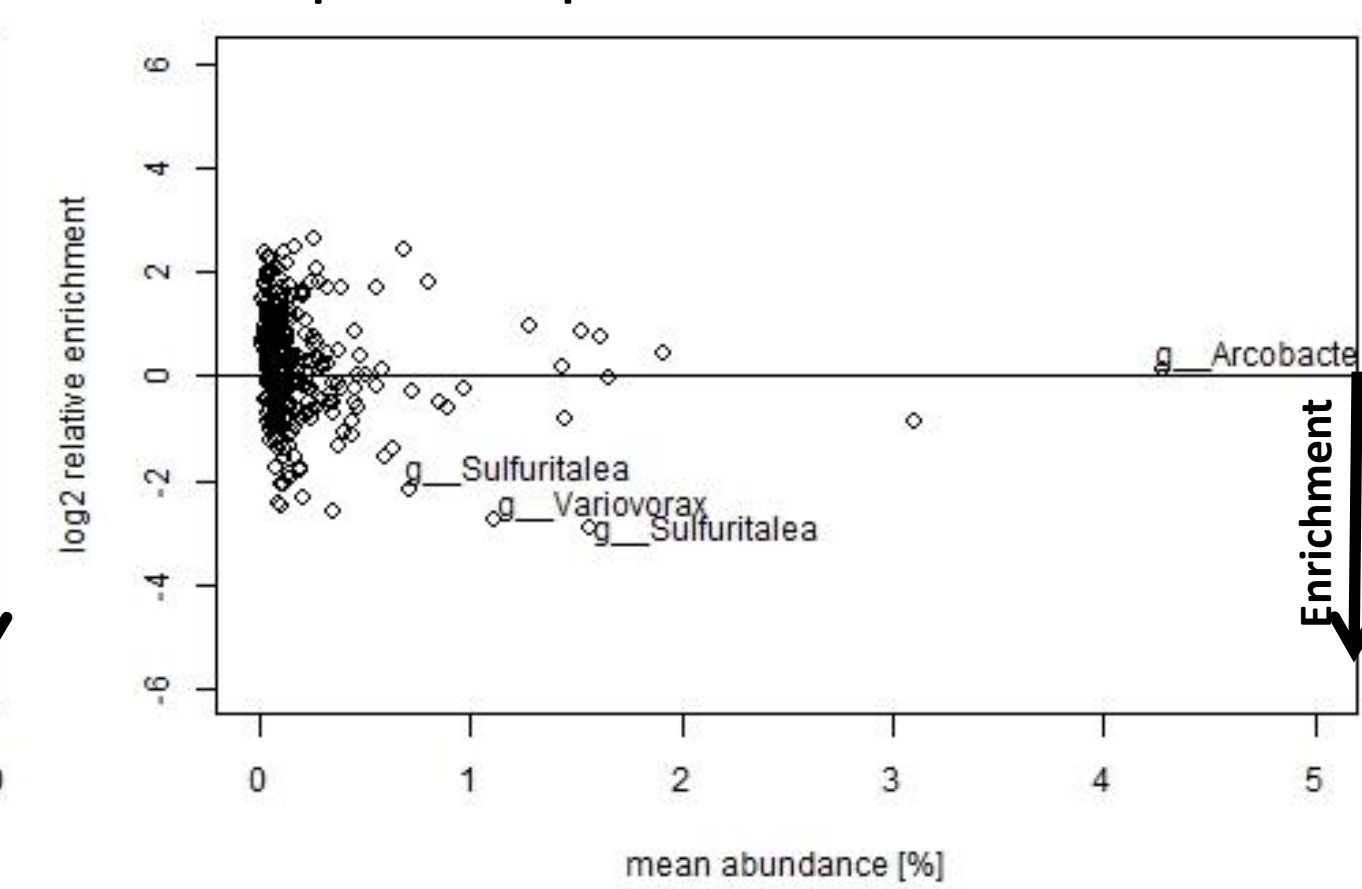
**Relative abundance of 10 most frequently occurring genera compared between the different sludge fractions.**  
The variation between the WWTPs is captured by the boxplot width.



### 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
1200	Pseudorhodobacter	0.7	1200	Pseudorhodobacter	0.8
1417	Gordonia	0.7	1417	Gordonia	0.8
8679	Fodiniibacter	0.7	203	Alicyclophus	0.7
1221	Streptococcus	0.7	1221	Streptococcus	0.8
5480	Nocardoides	0.6	11578	Variovorax	0.7
5643	Ferruginibacter	0.6	6976	Cloacibacterium	0.7
7902	Chitinophagaceae	0.6	4241	SMA-35	0.6
6268	Leucobacter	0.6	6930	HF_BF39	0.7
978	env_OPS_17	0.7	13925	Unclassified	0.7
1852	A0817	0.7	13230	Candidatus_Nitrospira	0.8
14179	Sulfuritalea	0.7	5387	Geothrix	0.8
6532	Ferruginibacter	0.7	3960	Betaproteobacteria	0.8
9728	Sulfuritalea	0.7	12821	Betaproteobacteria	0.8
13898	Candidatus_Epiflobacter	0.6	15181	Propionibacterium	0.6
8809	WCHB1-60	0.7	9372	PSB	0.6
13957	Caulobacteraceae	0.6	5960	Saccharomonas	0.6
5957	AKW1767	0.7	4338	Holophagaceae	0.8
6267	Rhodobates	0.7	4350	Unclassified	0.7
				env_OPS_17	0.6
				0319-6620	0.7

SHEAR SENSITIVITY					
Total sludge		Supernatant		Supernatant after shear	
OTU	taxonomic classification	R	OTU	taxonomic classification	R
995	Trichococcus	0.6	5179	Sulfuritalea	0.8
411	Chitinophagaceae	0.6	12129	Agromyces	0.6
201	Alicyclophus	0.6	3387	Geothrix	0.8
1581	Propionibacterium	0.7	1581	Nitrosomonas	0.6
1048	Propionibacterium	0.7	6536	OTU38909	0.7
9913	TM214	0.7	9913	TM214	0.8
1400	Propionibacterium	0.7	1400	Propionibacterium	0.8
184	Hydrogenobium	0.6	3235	Kinetoplastaceae	0.7
1012	Leucobacter	0.6	1012	Geothrix	0.8
2010	Hydrogenobium	0.6	13177	WCHB1-60	0.7
6010	Ferruginibacter	0.6	5430	Chloroflex	0.7
13957	Caulobacteraceae	0.6	13957	Rhodobacterium	0.7
3898	Trichococcus	0.6	3898	TM214	0.8
				env_OPS_17	0.6
				0319-6620	0.7

**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.