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Hansen, Susan Hove; Nierychlo, Marta; Larsen, Poul; Jørgensen, Mads Koustrup; Albertsen, Mads; Christensen, Morten Lykkegaard; Nielsen, Per Halkjær

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# Influence of microbial community composition on activated sludge floc properties

Susan H. Hansen, Marta Nierychlo, Poul Larsen, Mads K. Jørgensen, Mads Albertsen, Morten L. Christensen, Per H. Nielsen  
Center for Microbial Communities, Department Of Chemistry And Bioscience, Aalborg University, Denmark

## Background

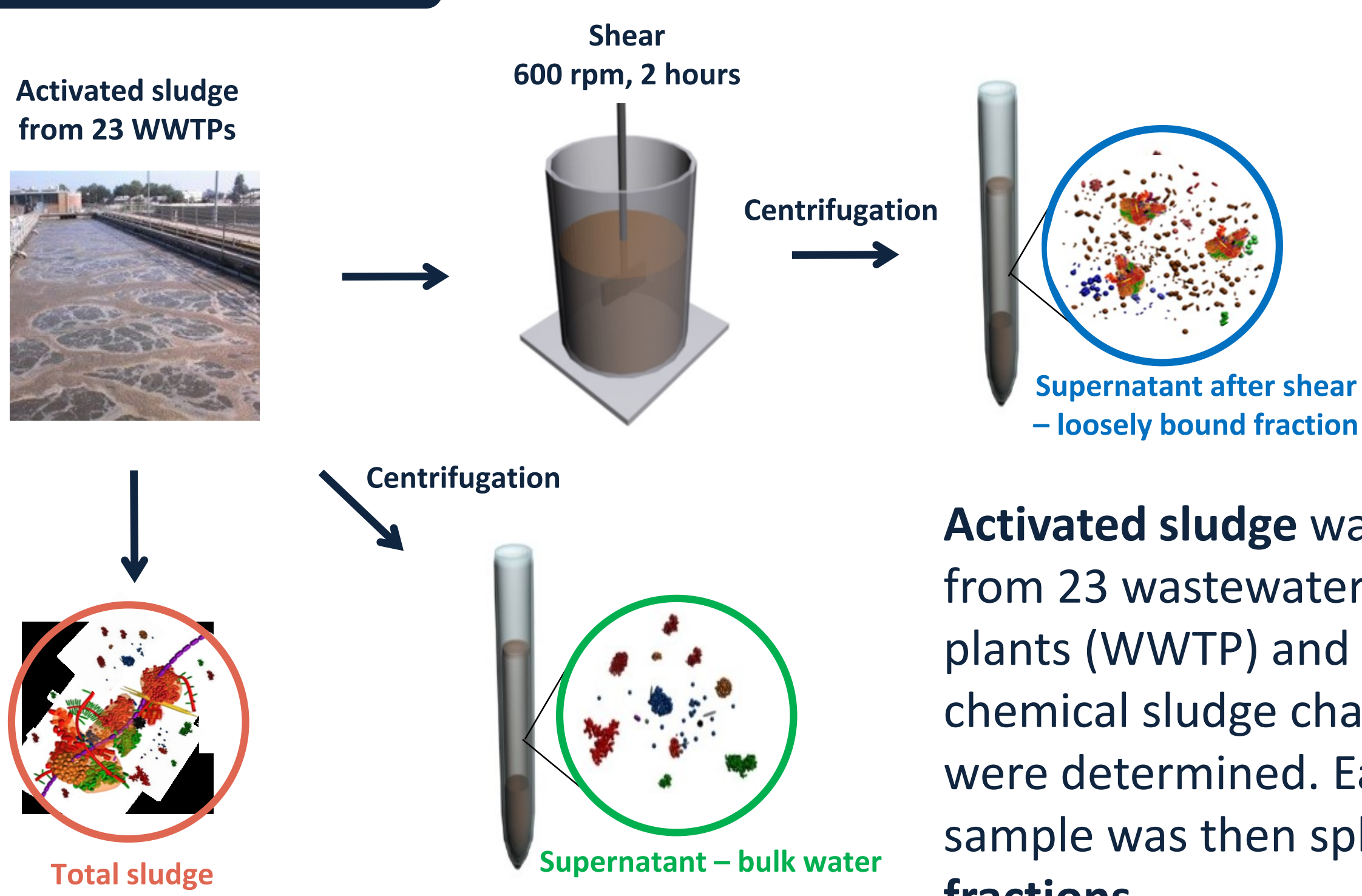
The activated sludge process is key in modern wastewater treatment. The activated sludge ecosystem contains a core community of abundant organisms and it must be stable in order to maintain the desired nutrient removal. In addition, bacterial morphology, mode of growth and extracellular polymeric substances (EPS) composition determine floc size, shape and strength, which in turn influence sludge properties that are important for plant operation. A few organisms have been correlated with different sludge properties, however, the picture is far from complete.

## Aim

- To investigate whether the microbial community composition differs between the flocs and the supernatant (bulk water) and whether certain bacterial species correlate to floc strength.

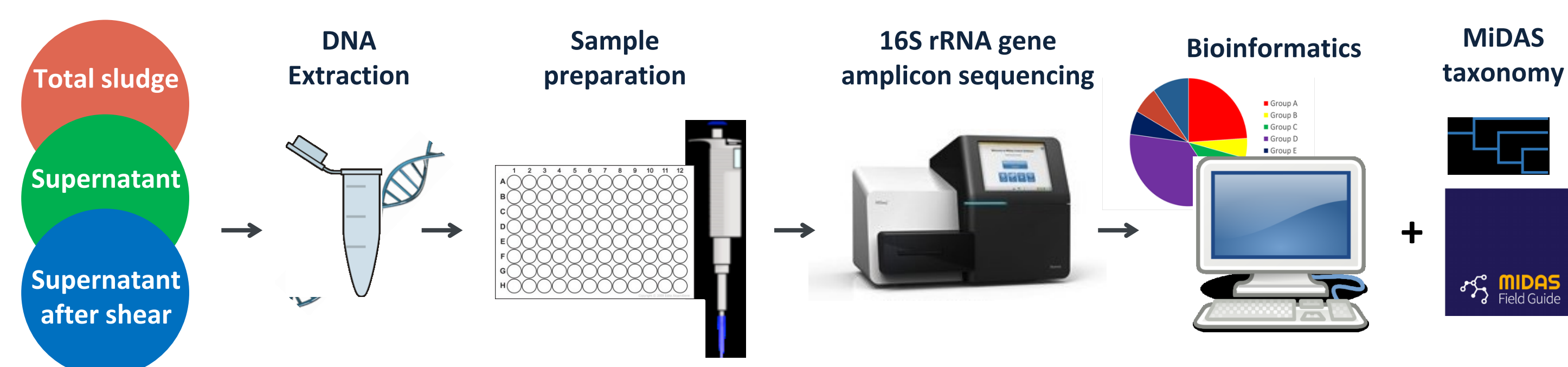
## Methods

### Sludge fractions



Activated sludge was collected from 23 wastewater treatment plants (WWTP) and physio-chemical sludge characteristics were determined. Each sludge sample was then split in three fractions.

### 16S amplicon sequencing



## Correlation between sludge parameters and bacteria in the sludge fractions

SLUDGE VOLUME INDEX				Supernatant after shear			
OTU	taxonomic classification	abundance [%]	R	OTU	taxonomic classification	abundance [%]	R
276	g_Lactococcus	0.1	0.8	11683	f_Caldilineaceae	0.9	0.6
9794	g_Arcobacter	0.2	0.8	11886	g_Caldilinea	0.2	0.7
11683	f_Caldilineaceae	3.2	0.7	12307	g_Pseudorhodobacter	0.2	0.7
11886	g_Caldilinea	0.1	0.7	4117	g_Gordonia	0.2	0.9
12307	g_Pseudorhodobacter	0.3	0.7	8679	g_Fodinibacter	0.2	0.9
4117	g_Gordonia	0.2	0.7	5480	g_Nocardioides	0.1	0.7
8679	g_Fodinibacter	0.3	0.7	10334	g_Thermomonas	0.1	0.7
13221	g_Streptococcus	0.1	0.7	8804	g_Alicyclophilus	4.6	0.8
5480	g_Nocardioides	0.1	0.6	13375	g_Parabacteroides	0.2	0.6
5643	g_Ferruginibacter	0.2	0.6	975	g_Mycobacterium	0.3	0.6
7904	f_Chitinophagaceae	0.3	0.6	15219	g_Brevundimonas	0.1	0.6
6268	g_Leucobacter	0.2	0.9	2634	g_Enterococcus	0.1	0.7
49	g_BS063	0.3	0.7	13221	g_Streptococcus	0.3	0.7
5387	g_Geothrix	0.4	-0.8	6930	g_HF_BF39	0.4	0.7
13371	g_Nannocystis	0.2	-0.7	2741	g_B1-K2-141	0.5	0.6
1858	g_A0837	0.3	-0.7	2681	g_MK04	0.2	0.6
14179	g_Sulfuritalea	0.4	-0.7	5384	f_Rhodobacteraceae	0.3	0.7
6532	g_Ferruginibacter	0.4	-0.7	954	f_Phyllobacteriaceae	0.3	0.6
9778	g_Sulfuritalea	0.5	-0.7	7086	f_Lachnospiraceae	0.1	0.8
13898	g_CandidatusEpiflobacter	0.7	-0.6	4378	f_Lachnospiraceae	0.1	0.6
15957	f_Caulobacteraceae	0.1	-0.8	5387	g_Geothrix	0.1	-0.8
5957	f_AKYH767	0.1	-0.7	12821	c_Betaproteobacteria	7.6	-0.8
6267	o_Rhizobiales	0.3	-0.7	196	f_env_OPS_17	0.3	-0.6
				10601	f_0319-6G20	0.1	-0.7

SHEAR SENSITIVITY				Supernatant after shear			
OTU	taxonomic classification	abundance [%]	R	OTU	taxonomic classification	abundance [%]	R
5954	g_Trichococcus	2.1	0.6	14179	g_Sulfuritalea	3.2	0.7
12837	g_Pseudacidovorax	0.3	0.7	7201	g_Bacteriovorax	0.1	0.6
10410	g_Proteinilasticum	0.2	0.7	6275	g_Tetrasphaera	1.5	-0.8
831	g_Thauera	0.2	0.8	13677	p_WCHB1-60	0.2	-0.7
14300	f_Propionibacteriaceae	0.1	-0.6	6532	g_Ferruginibacter	0.2	-0.7
194	g_Hyphomicrobium	0.9	-0.6	11480	g_rj14	0.2	-0.7
10225	f_Kineosporiaceae	0.2	-0.6				
2101	g_QEDR3BF09	0.3	-0.7				
6532	g_Ferruginibacter	0.4	-0.6				
13898	g_CandidatusEpiflobacter	0.7	-0.6				
3086	g_Tessaracoccus	0.1	-0.7				

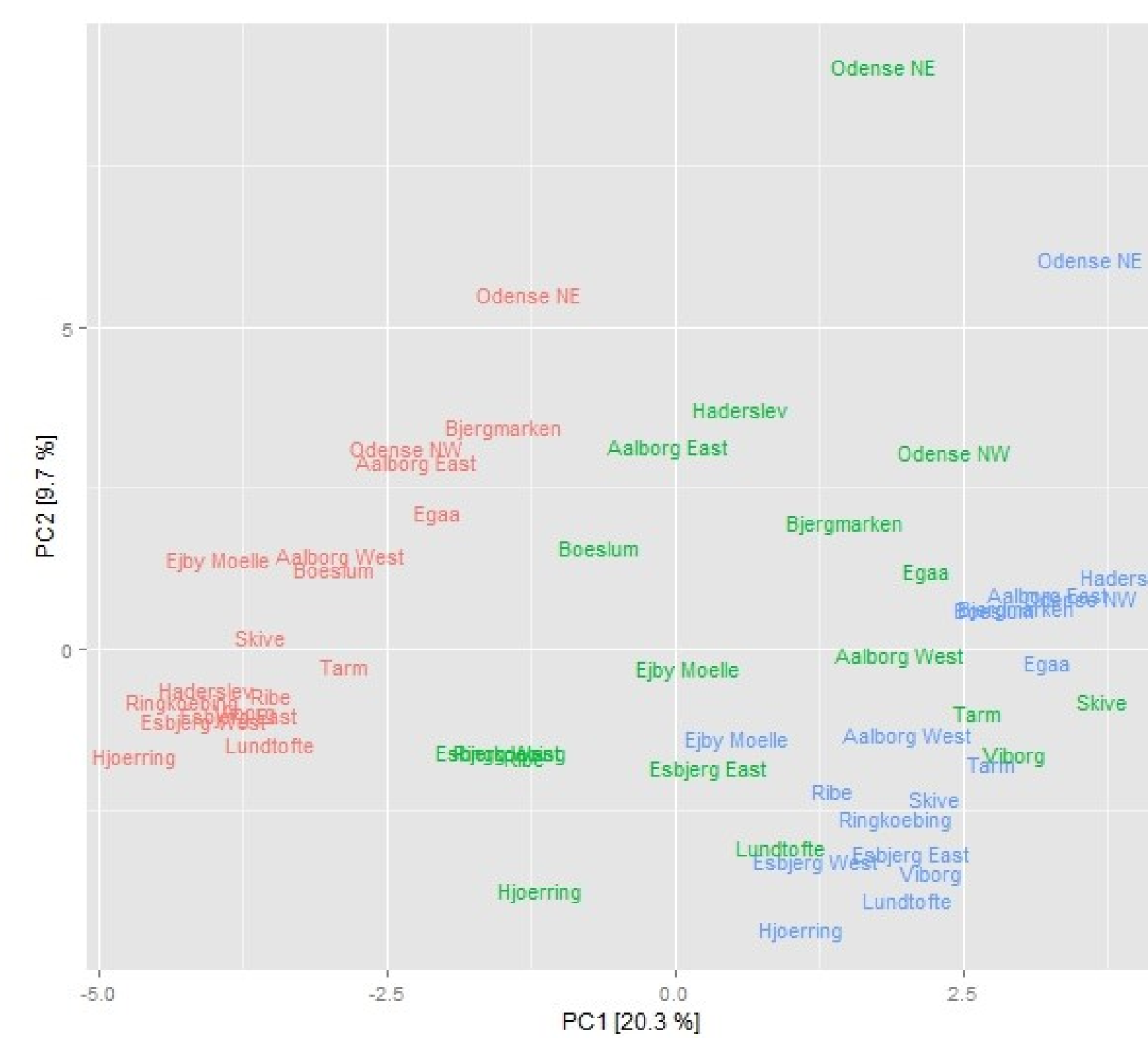
**Spearman correlation** of bacteria present in different sludge fractions with important sludge characteristics: Sludge Volume Index (SVI) and shear sensitivity. Bacteria that were strongly correlated ( $-0.6 > R > 0.6$ ) with the mentioned parameters are listed above. One abundant OTU, belonging to the class of Betaproteobacteria, seems to have a positive impact on SVI. High abundance of *Sulfuritalea* correlates with high shear sensitivity, whereas the presence of *Tetrasphaera* correlates to low shear sensitivity.

## Conclusions

- Microbial community composition relates to different activated sludge fractions.
- Acrobacter* and *Sulfuritalea* are enriched in the bulk water fraction and in the fraction loosely bound to the floc and could therefore be associated as poor floc formers.
- Dechloromonas* and *Tetrasphaera* abundances decreased in supernatants and could therefore be associated with potential strong floc formers.
- Sulfuritalea* and *Tetrasphaera* can be correlated to sludge characteristics that are important for the proper plant operation (SVI and shear sensitivity).

## Results

### Community composition relates to different activated sludge fractions from 23 WWTPs



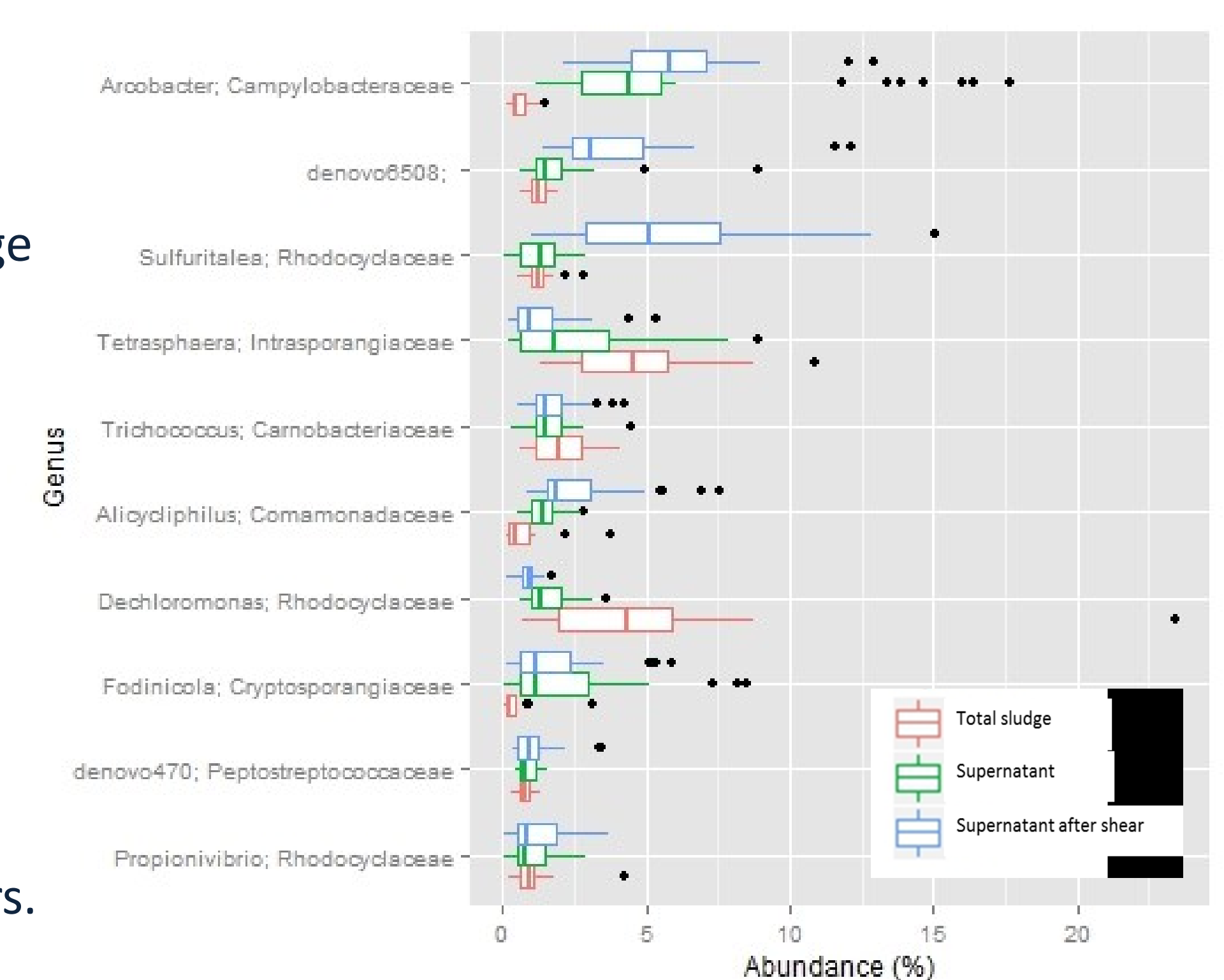
**Multivariate analysis** visualized the relationship between all samples analyzed from 23 WWTPs. Samples grouping into individual sludge fractions (total sludge – bulk water – loosely bound fraction) can be clearly observed. WWTP city names are presented.

- Total sludge
- Supernatant before shear
- Supernatant after shear

### Comparison of relative abundance between the different activated sludge fractions

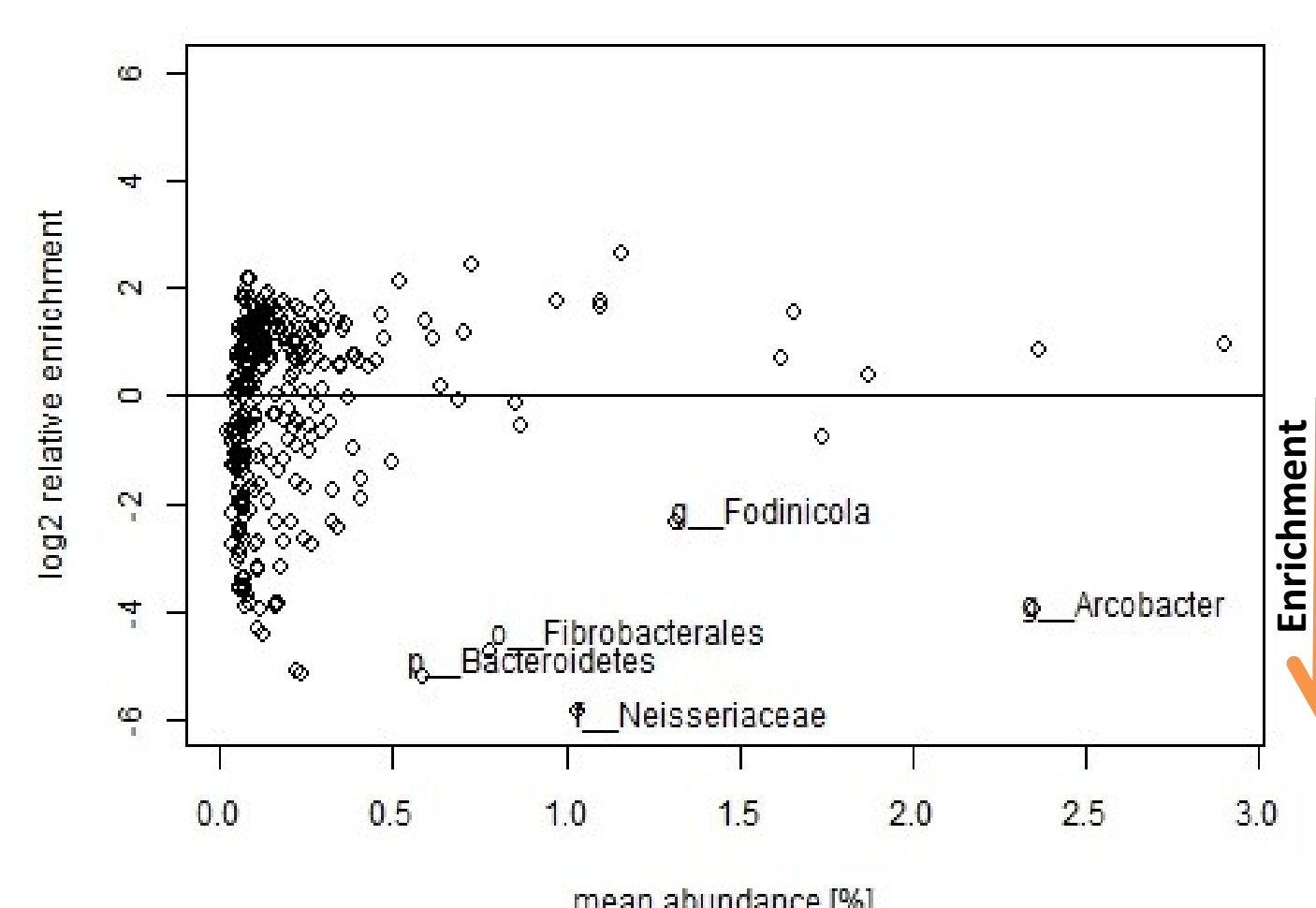
**Relative abundance** of 10 most frequently occurring genera compared between the different sludge fractions.

The variation between the WWTPs is captured by the boxplot width. *Acrobacter* seems to be linked to the loosely bound sludge fraction, whereas especially *Dechloromonas* and *Tetrasphaera* could be potential strong floc formers.



### Enrichment of bacteria in different activated sludge fractions

Bacteria enriched in the **supernatant** compared to **total sludge**. Highlighted bacteria seem to contribute to poor floc properties.



Bacteria enriched in the **supernatant after shear** compared to **supernatant**. Highlighted bacteria seem to be loosely attracted to the sludge flocs.

