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

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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

mni@bio.aau.dk

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

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.

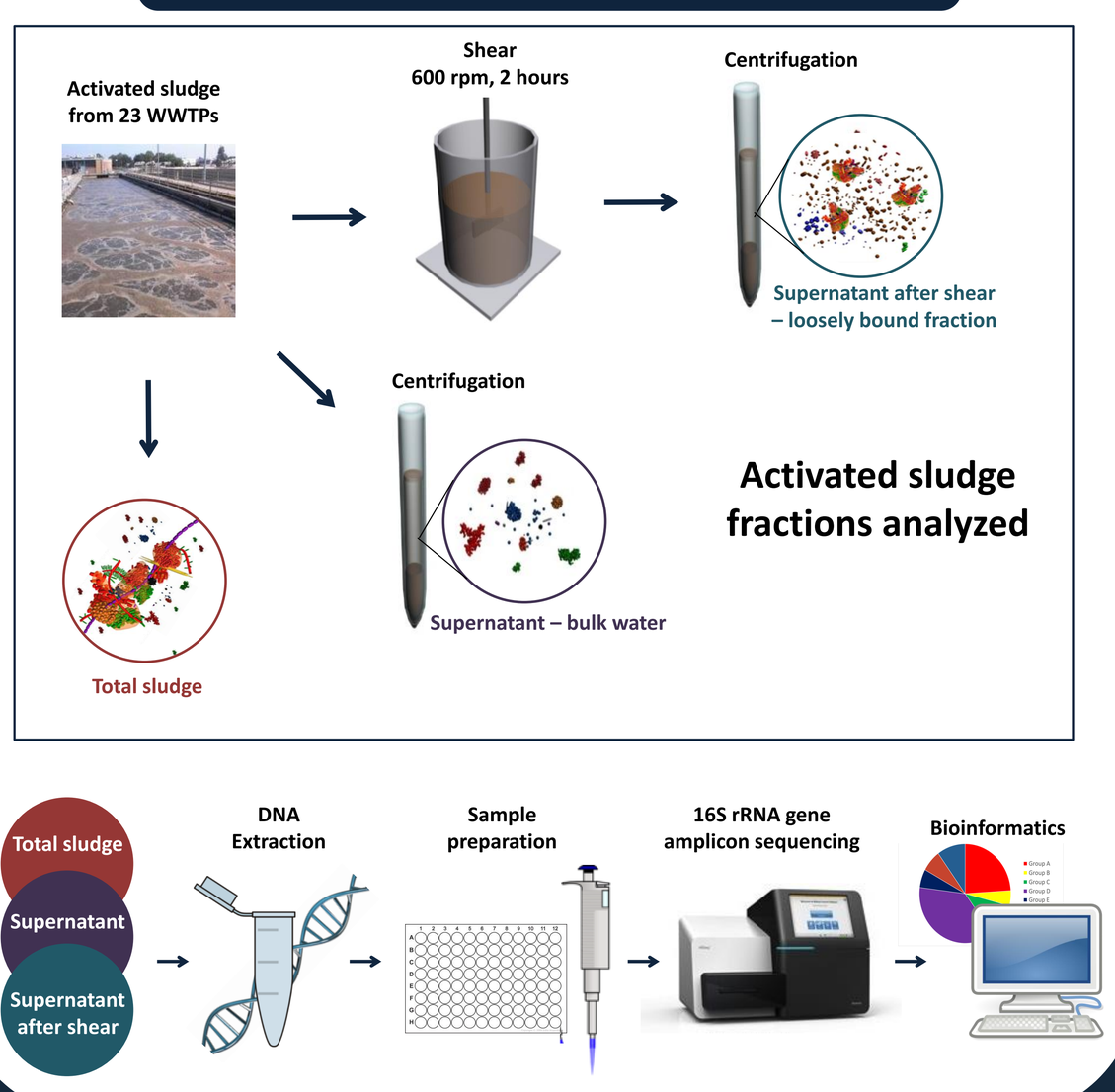
DNA-based identification of microorganisms using 16S rRNA gene amplicon sequencing has been developed over the past few years and is now ready to use for more comprehensive studies related to plant operation and optimization thanks to short analysis time, low cost, high throughput, and high taxonomic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



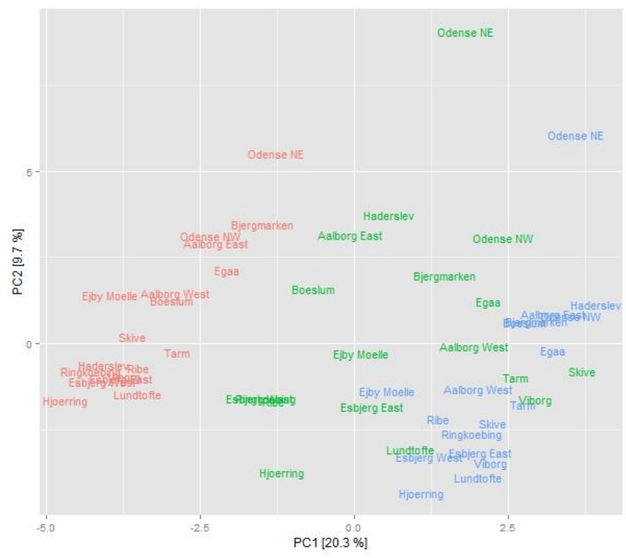
Conclusions

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.

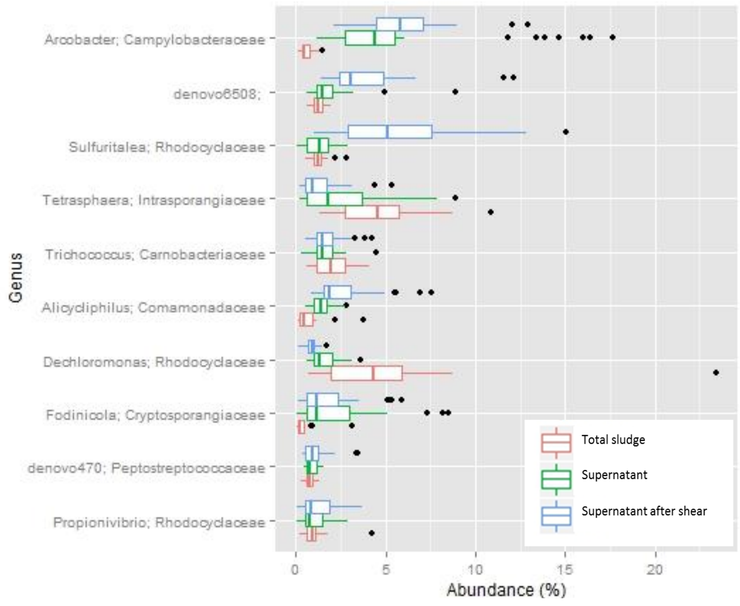
A number of bacterial species can be correlated to the sludge characteristics that are important for the proper plant operation (SVI, floc strength, and EPS content).

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

Results

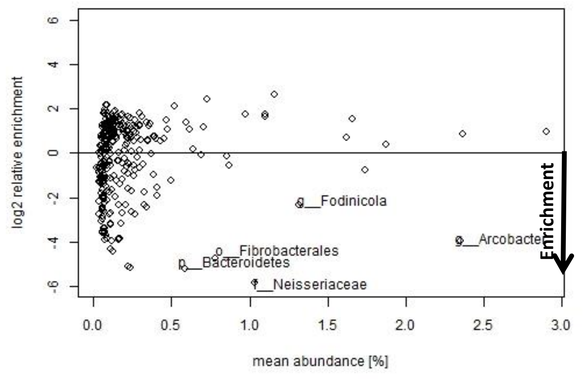


Community composition in different activated sludge fractions
The figure shows the relationship between all samples analyzed from 23 WWTPs.
Samples grouping into individual sludge fractions (total sludge – bulk water – loosely bound fraction) can be clearly observed.

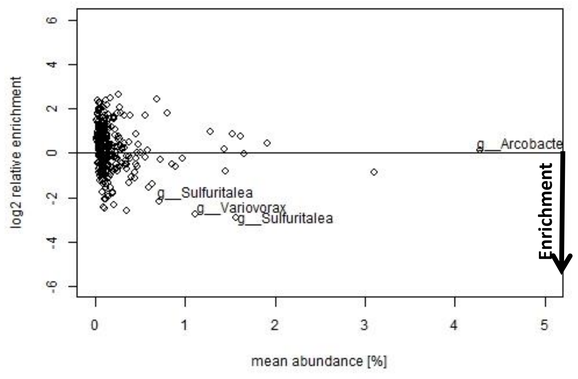


Relative abundance
of 10 most frequently occurring genera compared between the different sludge fractions .
The variation between the WWTPs is captured by the boxplot width.

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	OTU	taxonomic classification	R
276	g__Lactococcus	0.8	276	g__Lactococcus	0.7	11683	f__Caldilineaceae	0.6
9794	g__Arcobacter	0.8	6323	g__Arcobacter	0.6	11886	g__Caldilinea	0.7
11683	f__Caldilineaceae	0.7	11683	f__Caldilineaceae	0.8	6545	g__Caldilinea	0.6
11886	g__Caldilinea	0.7	8679	g__Fodinibacter	0.8	12307	g__Pseudorhodobacter	0.7
12307	g__Pseudorhodobacter	0.7	13221	g__Streptococcus	0.8	4117	g__Gordonia	0.9
4117	g__Gordonia	0.7	10334	g__Thermomonas	0.7	8679	g__Fodinibacter	0.9
8679	g__Fodinibacter	0.7	203	g__Alicycliphilus	0.7	5480	g__Nocardioides	0.7
13221	g__Streptococcus	0.7	15756	f__Nocardioidaceae	0.6	10334	g__Thermomonas	0.7
5480	g__Nocardioides	0.6	11578	g__Variovorax	0.7	8804	g__Alicycliphilus	0.8
5643	g__Ferruginibacter	0.6	6976	g__Cloacibacterium	0.7	13375	g__Parabacteroides	0.6
7904	f__Chitinophagaceae	0.6	4241	o__SHA-35	0.6	14340	g__Aeromicrobium	0.6
6268	g__Leucobacter	0.9	6930	g__HF_BF39	0.7	975	g__Mycobacterium	0.6
978	f__env.OPS_17	0.7	13925	p__Unclassified	0.7	15219	g__Brevundimonas	0.6
49	g__BS063	0.7				13530	g__Candidatus_Nitrotoga	0.9
						6763	g__Rhodovulum	0.8
						2634	g__Enterococcus	0.7
						13221	g__Streptococcus	0.7
						6930	g__HF_BF39	0.7
5387	g__Geothrix	-0.8				2741	g__B1-K2-141	0.6
8210	g__Geothrix	-0.7				2681	g__MK04	0.6
13371	g__Nannocystis	-0.7				5384	f__Rhodobacteraceae	0.7
2357	g__Propionivibrio	-0.9				954	f__Phyllobacteriaceae	0.6
15181	g__Propioniciclava	-0.6				7086	f__Lachnospiraceae	0.8
1858	g__A0837	-0.7				4378	f__Lachnospiraceae	0.6
14179	g__Sulfuritalea	-0.7	5387	g__Geothrix	-0.8	5387	g__Geothrix	-0.8
6532	g__Ferruginibacter	-0.7	3960	c__Betaproteobacteria	-0.7	12821	c__Betaproteobacteria	-0.8
9778	g__Sulfuritalea	-0.7	12821	c__Betaproteobacteria	-0.9	13371	g__Nannocystis	-0.6
13898	g__CandidatusEpiflobacter	-0.6	15181	g__Propioniciclava	-0.6	1821	g__Leadbetterella	-0.9
8809	p__WCHB1-60	-0.7	9172	g__P58	-0.6	5787	g__H106	-0.7
15957	f__Caulobacteraceae	-0.8	5969	g__Saccharimonas	-0.6	9302	g__Thiothrix	-0.6
5957	f__AKYH767	-0.7	4338	f__Holophagaceae	-0.8	196	f__env.OPS_17	-0.6
6267	o__Rhizobiales	-0.7	4250	p__Unclassified	-0.7	10601	f__0319-6G20	-0.7

SHEAR SENSITIVITY

Total sludge			Supernatant			Supernatant after shear		
OTU	taxonomic classification	R	OTU	taxonomic classification	R	OTU	taxonomic classification	R
5954	g__Trichococcus	0.6	14179	g__Sulfuritalea	0.6	14179	g__Sulfuritalea	0.7
4341	f__Christensenellaceae	0.6	12376	f__Saprospiraceae	0.6	11231	p__BD1-5	0.6
203	g__Alicycliphilus	0.6	5387	g__Geothrix	0.6	7201	g__Bacteriovorax	0.6
12837	g__Pseudaciovorax	0.7	4484	g__Iamia	0.7			
15181	g__Propioniciclava	0.7	11676	g__Nitrosomonas	0.8			
10410	g__Proteiniclasticum	0.7	6553	g__QEDR3BF09	0.7			
831	g__Thauera	0.8	9911	f__TM214	-0.7	9911	f__TM214	-0.8
9911	f__TM214	-0.7	14300	f__Propionibacteriaceae	-0.6	14300	f__Propionibacteriaceae	-0.6
14300	f__Propionibacteriaceae	-0.6	11684	g__Hyphomicrobium	-0.7	11684	g__QEDR3BF09	-0.7
194	g__Hyphomicrobium	-0.6	10225	f__Kineosporiaceae	-0.7	6275	g__Tetrasphaera	-0.8
10225	f__Kineosporiaceae	-0.6	6275	g__Tetrasphaera	-0.7	13677	p__WCHB1-60	-0.7
2101	g__QEDR3BF09	-0.7	13677	p__WCHB1-60	-0.7	6532	g__Ferruginibacter	-0.7
6532	g__Ferruginibacter	-0.6	5435	p__Chloroflexi	-0.7	11480	g__rJ14	-0.7
13898	g__CandidatusEpiflobacter	-0.6	6763	g__Rhodovulum	-0.7	1742	g__LF_BF07	-0.7
3086	g__Tessaracoccus	-0.7	6551	g__Rhizobacter	-0.6	7842	g__35-7A	-0.7

DEGREE OF FLOCCULATION
turbidity / mean floc size

Total sludge			Supernatant			Supernatant after shear		
OTU	taxonomic classification	R	OTU	taxonomic classification	R	OTU	taxonomic classification	R
			9770	g__Prevotella	0.6	11683	f__Caldilineaceae	0.6
			3046	g__Competibacter	0.6	3465	p__Unclassified	0.7
			5387	g__Geothrix	-0.7	15756	f__Nocardioidaceae	0.7
			3900	g__Zoogloea	-0.7			
			13371	g__Nannocystis	-0.7	5387	g__Geothrix	-0.7
12376	f__Saprospiraceae	-0.7	1512	g__Stella	-0.6	13371	g__Nannocystis	-0.7

CONDITIONS FOR FLOCCULATION
total EPS / cations

Total sludge			Supernatant			Supernatant after shear		
OTU	taxonomic classification	R	OTU	taxonomic classification	R	OTU	taxonomic classification	R
4341	f__Christensenellaceae	0.6	4341	f__Christensenellaceae	0.7	10410	g__Proteiniclasticum	0.7
10410	g__Proteiniclasticum	0.6	10410	g__Proteiniclasticum	0.7	4831	g__Actinobaculum	0.6
12676	o__PeM15	0.6				15498	f__Neisseriaceae	0.6
12676	o__PeM15	0.6				7535	p__Unclassified	0.6
5610	g__Run-SP154	0.7				5435	p__Chloroflexi	-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.