Can DNA sequencing show differences between microbial communities in Polish and Danish wastewater treatment plants?

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ABSTRACT

The microbial populations in the activated sludge of two Polish wastewater treatment plants (WWTPs) were identified and quantified using Illumina sequencing of 16S ribosomal RNA amplicons over a 2-year period. Their dynamics over time were compared to Danish WWTPs (data collected in previous studies by Center for Microbial Communities, Aalborg University). The bacterial communities in Polish and Danish WWTPs were similar to each other, but the microbial diversity in Polish WWTPs was lower. The dominant genera in Polish WWTPs were more abundant than in Danish WWTPs; 30 of them constituted more than half the of activated sludge community. Polish WWTPs showed a higher abundance of bacteria involved in nitrogen and chemical oxygen demand removal (*Proteobacteria* and *Bacteroidetes*), while polyphosphate-acculumating bacteria were the dominant bacterial group in Danish plants. The microbial community structures in the examined Polish WWTPs were relatively similar to each other and showed strong seasonal variations which are not normally observed in Danish WWTPs.

Key words | 16S rRNA amplicon sequencing, activated sludge, bacterial populations, molecular biology

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INTRODUCTION

Knowledge of the microbiology of activated sludge (AS) communities reached its current level owing to methods that were culture- independent (Nielsen *et al.* 2012; Sheik *et al.* 2014). In previous studies focused on microbial population dynamics in the AS of Polish wastewater treatment plants (WWTPs), the identity and quantity of bacteria were determined using fluorescence *in situ* hybridization (FISH) (Miłobędzka & Muszyński 2015; Miłobędzka *et al.* 2016). Meanwhile, the unprecedented decrease in sequencing costs and increased read length (Caporaso *et al.* 2012) has made DNA-based methods for phylogenetic analysis more available and reliable. Therefore it is advisable to use more advanced, modern techniques, such as DNA extraction, sequencing library preparation and sequencing.

Studying AS biodiversity requires not only observing changes in the abundance of microbial populations connected with their metabolic functions, but also testing which populations are frequent enough to be important key players in carbon and nutrients turnover in a WWTP. As microbial diversity is enormous (Quince *et al.* 2008), only a small part of the AS can be functionally characterized *in situ*. A Danish research group (Saunders *et al.* 2016) decided to use the core community concept (Grime 1998; Gibson *et al.* 1999) to distinguish putatively important microbial groups, based on the assumption that the relative carbon removal activity of microorganisms should be mirrored in their relative abundance. Illumina sequencing of 16S ribosomal RNA amplicons of the V4 region was applied to investigate microbial communities in 13 Danish WWTPs with nutrients removal. Saunders *et al.* (2016) stated that there is a core community of 63 abundant genus-level operational taxonomic units (OTUs), constituting up to 68% of the total reads.

Prior to our work reported here, no Polish WWTPs had a reliable database of bacterial abundance measured with molecular methods. In the presented study, microbial populations in the AS of two Polish WWTPs were studied over a 2-year period using DNA sequencing to find the most abundant bacterial genera, and to analyse their potential functions in the system, as well as to investigate their

doi: 10.2166/wst.2017.015

dynamics over time, and to check whether or not they are included in the core community from Danish WWTPs.

MATERIALS AND METHODS

Sampling and WWTP data

Both Polish WWTPs treat domestic wastewater and they have similar sizes (73,400 and 76,000 population equivalents for WWTP I and II, respectively). Both plants have primary clarifiers, biological N-removal (nitrification and denitrification) and well-defined enhanced biological phosphorous removal steps; however, iron-based coagulants (PIX) are additionally used for P-precipitation. The nitrification tanks are aerated with fine bubble diffusers. The investigated plants differ in reactors' configuration and main technological parameters of the wastewater treatment process. WWTP I has an anaerobic–anoxic–aerobic (A2O) type of reactor, a predenitrification tank and a fermenter. WWTP II uses the University of Cape Town (UCT) configuration. During the study severe sludge bulking and poor N-removal (mostly in winter) were documented in WWTP I, while in WWTP II seasonal operational problems were mostly connected with the settling and foaming of AS. Detailed description of both plants' configurations and performance can be found in the supplementary material (available with the online version of this paper).

Grab samples of AS (100 mL) were collected twice a year in the period 2012–2014 (at the beginning of March and at the end of September) from the aerobic process tanks (sampling depth was over 30 cm below wastewater surface), and kept on ice until DNA extraction.

Molecular analysis

DNA sequencing (carried out by DNAsense ApS (Aalborg, Denmark)) was used to count the number of 16S rRNA reads from each bacteria in the sample, which served as an estimate of the abundance of the bacterial species. DNA was isolated with a PowerSoil® DNA isolation kit; the obtained DNA (not additionally concentrated) was stored at −80 °C until analyses. 16S rRNA amplicon library preparation procedures targeting the V1-3 variable regions were performed according to the method of Caporaso *et al.* (2012), using primers adapted from the Human Gut Consortium (Ward *et al.* 2012). Polymerase chain reactions, DNA sequencing and 16S rRNA amplicon bioinformatic processing were run using the method of Albertsen *et al.* (2015). DNA sequencing included collecting the purified sequencing libraries in equimolar concentrations and diluting them to 4 nM. Investigated samples were paired end sequenced (2 × 301 bp) on a MiSeq (Illumina) using a MiSeq Reagent kit v3, 600 cycles (Illumina) following the standard guidelines for preparing and loading samples on the MiSeq. Also 10% Phix control library was spiked in to overcome low complexity issue often observed with amplicon samples.

Statistical measures and methods

Standard statistical analyses, parametric correlation analyses, Student's *t*-test for independent samples and principal component analysis (PCA) were carried out using R Statistical Software (Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Identity, abundance and membership of the core community

The dominant bacteria belonged to phyla *Proteobacteria* and *Bacteroidetes*; their mean abundance (in percentage of pair of reads) in the AS of the tested WWTPs is shown in Figure 1, seasonal variations are shown in Figure 2. The 30 most abundant bacterial groups constituted in total more than half of the microbial community, with the average abundance for each group being within the range from 0.7 to 9%. The broad group name (phylum) and a specific name (genus), or the OTU number (if no genus name could be assigned), are listed in Figure 1.

The dominant denitrifying heterotrophic bacteria *Rhodoferax* reached almost 20% abundance in one sampling period (range 1%–19.2%, 9% was the mean value for Polish WWTPs). The second most abundant group, *Flavobacterium,* accounted for 3.8% and 5.1% in WWTP I and II, respectively (range 0.6%–12%, average abundance in Polish WWTPs – 4.5%). This genus and three other abundant OTUs from phylum *Bacteroidetes* (QEDR3BF09 (3.1%), PHOS–HE28 (2.6%), CYCU–0281 (2%)) have not yet been assigned specific functions in the AS microbial community.

Most of the genera (18 described and 5 uncharacterized OTUs) found in the AS of Polish WWTPs belonged to the core community described by Saunders *et al.* (2016). They constituted on average 43.1% of pairs of reads. Seven other genera constituted on average 8.4% of pairs of reads and belonged to families *Anaerolinaceae* (SBR 1029), *Bdellovibrionaceae*

	FIL	AOB	NOB	PAO	GAO	HET	DN	Mean abundance in WWTP: Danish: domestic	Danish: mixed	Danish: industrial	Polish: WWTP I	Polish: WWTP II
Proteobacteria; Rhodoferax								2.5	1.5	0.4	12.7	5.3
Bacteroidetes; Flavobacterium								0.5	0.2	0.9	3.8	5.1
Bacteroidetes; QEDR3BF09								1.3	1.2	0.1	2.1	4.1
Bacteroidetes; PHOS−HE28								0.8	0.9	0.7	2.1	3
Bacteroidetes; CYCU−0281								0.9	0.9	0.5	1.8	2.1
Bacteroidetes; Candidatus Epiflobacter								0.9	0.8	0.1	1.9	1.8
Bacteroidetes; PHOS−HE31								0.8	0.6	0.3	2.4	1
Proteobacteria; Dechloromonas								1.5	0.4	0.1	1.5	1.8
Proteobacteria; Sulfuritalea								0.5	0.5	0.1	2.2	1.1
Proteobacteria; 188up								0.5	0.3	0	1.6	1.3
Bacteroidetes; OTU_306								0	0.1	0.1	0.8	2.1
Chloroflexi; SBR1029								0.8	1	0.2	1.6	1.3
Proteobacteria; Candidatus Accumulibacter								0.2	0.2	0.3	1.6	1.2
Proteobacteria; Arcobacter								0.2	0.1	0.1	2	0.8
Bacteroidetes; OTU_371								0	0	0	1.3	1.3
Proteobacteria; Ferribacterium								0.4	0.7	0.1	1.3	1.3
Proteobacteria; OM27 clade								0.3	0.2	0.1	1	1.6
Proteobacteria; Aquabacterium								0.2	0.1	0.1	1.2	1.4
Bacteroidetes; Ferruginibacter								0.9	0.7	0.3	0.6	1.9
Bacteroidetes; MK04								0.7	0.9	0.1	2.1	0.3
Proteobacteria; OTU_34								0.3	0.3	0.1	0.9	1.4
Bacteroidetes; OTU_423								0.1	0.1	0	0.7	1.2
Proteobacteria; Zoogloea								0.3	0.7	0.5	0.6	1.2
Nitrospirae; Nitrospira								0.8	0.7	0.3	1	0.7
Proteobacteria; Leptothrix								0.6	0.6	0.2	0.6	1
Proteobacteria; OTU_35								0.2	0.2	0.1	0.5	1.1
Proteobacteria; Simplicispira								0.6	0.6	0.1	1.3	0.2
Proteobacteria; Rhodobacter								2.3	2	0.8	0.8	0.6
Proteobacteria; Candidatus Nitrotoga								0.2	0.1	0.1	0.8	0.6
Proteobacteria; Nitrosomonas								0.4	0.4	0.3	0.6	0.7

Tested positive in published research papers
Can be both positive and negative
Tested negative in published research papers
No published research available

AOB: Ammonium oxidizing bacteria. involved in nitrogen removal. NOB: Nitrite oxidizing bacteria, involved in nitrogen removal. PAO: Polyphosphate accumulating bacteria, involved in biological phosphorus removal. GAO: Glycogen accumulating bacteria, can have a negative effect on biological phosphorus removal. HET: Heterotrophic bacteria, generally involved in COD removal. DN: Denitrifying bacteria, involved in nitrogen removal. FIL: Filamentous bacteria.

Figure 1 | The function, identity and abundance of the 30 most abundant bacteria

(OM27 clade), *Campylobacteraceae* (*Arcobacter*), *Rhodocyclaceae* (*Ferribacterium*, *Zoogloea*), *Nitrospiraceae* (*Nitrospira*) and *Gallionellaceae* (*Candidatus* Nitrotoga).

Seasonal variations and the influence of the WWTP on bacterial abundance

Temporal variations can be clearly seen in significant changes in abundance of 13 groups from 30 dominant genera (listed in Figure 2, higher abundance after winter and summer are marked with dark and light grey, respectively). The abundance of QEDR3BF09 (a group of *Saprospiraceae* that has not been investigated so far), *Candidatus* Accumulibacter (proteobacterial heterotrophic, denitrifying polyphosphate acculumating bacteria), clade OM27 and SBR1029 (filamentous *Chloroflexi*) increased after summer (light grey in Figure 2). The proteobacterial *Rhodoferaxx, Arcobacter, Dechloromonas*, *Rhodobacter*,

		Protobacteria; Rhodoferax	Bacteroidetes; Flavobacterium	Bacteroidetes; QEDB3BF09	Bacteroidetes; PHOS–HE28	Bacteroidetes; CYCU–0281	Bacteroidetes; Candidatus Epilithobacter	Bacteroidetes; PHOS–HE31	Protobacteria; Dechloromonas	Protobacteria; Sulfuritalea	Protobacteria; 188up	Bacteroidetes; OTU_306	Chloroflexi; SBR1029	Protobacteria; Candidatus Accumulibacter	Protobacteria; Arcobacter	Bacteroidetes; OTU_371	Protobacteria; Fermbacterium	Protobacteria; OM27 clade	Protobacteria; Aquabacterium	Bacteroidetes; Ferruginibacter	Bacteroidetes; MK04	Protobacteria; OTU_34	Bacteroidetes; OTU_423	Protobacteria; Zoogloea	Nitrospirae; Nitrospira	Protobacteria; Leptothrix	Protobacteria; OTU_35	Protobacteria; Simplicispira	Protobacteria; Rhodobacter	Protobacteria; Candidatus Nitrotoga	Protobacteria; Nitrosomonas	Number of reads after sequencing
WWTP I	Mar 2012	18.40	3.8	1.6	2.8	1.4	2.1	3.6	2.6	0.6	1.8	0.4	0.8	0.7	1.6	1.7	1.1	0.3	1.1	0.8	0.7	0.7	0.3	1.1	0	0.4	0.2	1.4	1.3	0.1	0.1	12614
	Sep 2012	3.6	0.6	3.6	1.2	2.9	4.7	1.4	1.6	1.8	2.5	0.6	1.8	1.5	0.8	0.5	2.1	1.2	1	0.6	0.5	2.1	0.5	0.3	3.6	0.5	0.4	0.4	1.1	0.4	0.2	0.6	15302
	Mar 2013	17.70	4.4	2.1	3	0.9	0.9	4	0.3	3.3	1.6	1.8	0.3	1.3	3.1	2.9	0.1	0.2	1	0.9	0.5	0.8	1.2	0.9	0.3	0.5	0.5	0.7	1.5	0.7	1.6	0.8	16615
	Sep 2013	4.5	2.8	1.8	1.3	2.2	1.3	1.2	1.8	3.3	1.1	0.3	5	2	1.6	0.2	2.7	2.8	0.9	0.5	0.5	0.9	1.4	1.2	0.5	0.8	0.5	0.4	0.7	0.4	0.1	1	15851
	Mar 2014	19.20	7.5	1.4	1.9	1.4	0.5	1.9	1.4	1.7	1.2	0.7	0.3	2.6	2.8	1.4	0.5	0.5	1.9	0.4	0.5	1.5	1.7	0.5	0.5	1.1	0.2	1.5	0.7	0.9	0.7	0.4	9488
WWTP II	Mar 2012	8.8	12	2	2.6	2.4	4.3	1.6	1.6	1.4	2.8	1.2	0.9	0.8	1.2	1.4	0.3	0.5	0.7	0.4	0.5	0.6	1.7	0.8	1	0.4	1.8	0.2	0	0.9	0	0.8	9218
	Sep 2012	1	0.6	4.5	2.2	2.2	0.8	1.5	1.2	0.7	1.7	0.6	2.2	1.7	0.2	0.1	2	1.1	0.4	2.3	0	1	2.1	1.6	2	2.6	1	0	0	0.2	0	0.2	15054
	Mar 2013	8.6	7.3	1.1	4.9	2.6	1.4	0.6	3.1	0.9	0.9	2.7	0.5	0.7	1.4	3.3	2.1	1.4	0.8	1.3	0.1	1.6	0.8	0.8	0.1	0.4	1.2	0.6	0.9	1.9	0.4	0.4	7729
	Sep 2013	1.8	0.9	7.5	1	1.7	1.3	0.6	0.7	1.7	0.4	0.5	1.6	1.9	0.4	0	0.2	2.6	3.9	1.9	0.1	1.3	0.8	0.5	0.4	1.3	0.7	0	0.3	0	0.7	0.7	13006
	Mar 2014	6.5	4.8	5.6	4.4	1.6	1.4	0.6	2.5	0.9	0.7	4.4	1.1	0.9	0.9	1.6	2.1	2.4	1.1	2.1	0.5	2	0.5	1.1	0.5	0.5	0.8	0.1	0.7	1.1	1.5	1.1	11889

Figure 2 | Seasonal variations in abundance of 30 dominant bacteria in tested Polish WWTPs. Higher abundance after winter and summer are marked with dark and light grey, respectively

Simplicispira, *Candidatus* Nitrotoga and, in *Bacteroidetes*, *Flavobacterium*, PHOS–HE28 and OTU_371 presented the opposite trend (proliferation after winter, dark grey in Figure 2).

The abundance of bacteria of the same genus from two WWTPs were treated as two independent data sets, collected independently of one another. Student's *t*-test (for independent samples) was used to compare these two sets of quantitative data. The influence of the WWTP was significant only in the case of *Ferruginibacter* (*Chitinophagaceae*, *Bacteroidetes*; significance level 0) and could be also meaningful for MK04 (*Saprospiraceae*, *Bacteroidetes*; significance level 0.087) and OTU306 (*Bacteroidetes*; significance level 0.093).

Correlations between bacterial abundance

Parametric and non-parametric mutual correlations between bacterial groups were searched for with Spearman's and Pearson's coefficients, respectively. The alpha level has been set for 0.05 and obtained *p*-values were less than or equal to the alpha for relations listed below.

Strong linear relations were observed between OTU35 and *Aquabacterium* ($r = 0.837$) and also between *Simplicispira* and PHOS–HE31 ($r = 0.820$). Strong parametric and non-parametric correlations ($r = 0.841$ and 0.866, respectively) also connected *Rhodoferax* and OTU371.

The abundance of *Rhodobacter* was very strongly parametrically and non-parametrically correlated with the abundance of bacteria from genera OTU371 (parametric $r = 0.925$, non-parametric $r = 0.953$,) and *Rhodoferax* (parametric $r = 0.905$, non-parametric $r = 0.920$). There was also a strong nonlinear correlation between *Rhodobacter* and PHOS–HE31 ($r = 0.869$). A similar relationship was found between *Ferribacterium* and *Chloroflexi* SBR1029 ($r = 0.800$).

A strong, non-parametric relation was observed between PHOS–HE31 and *Rhodoferax* ($r = 0.816$). The only negative, non-parametric relationship was noticed between OTU371 and OM27clade (-0.831).

PCA analyses

PCA of the microbial communities in all 10 samples (Figure 3) showed that the bacterial populations in the AS of the Polish WWTPs were distinguished by sampling period (even with a gap of up to 3 years between the samples) and also by plant. Samples from March shown by circles are on the lower right part of Figure 3, while squares presenting samples from September are in the

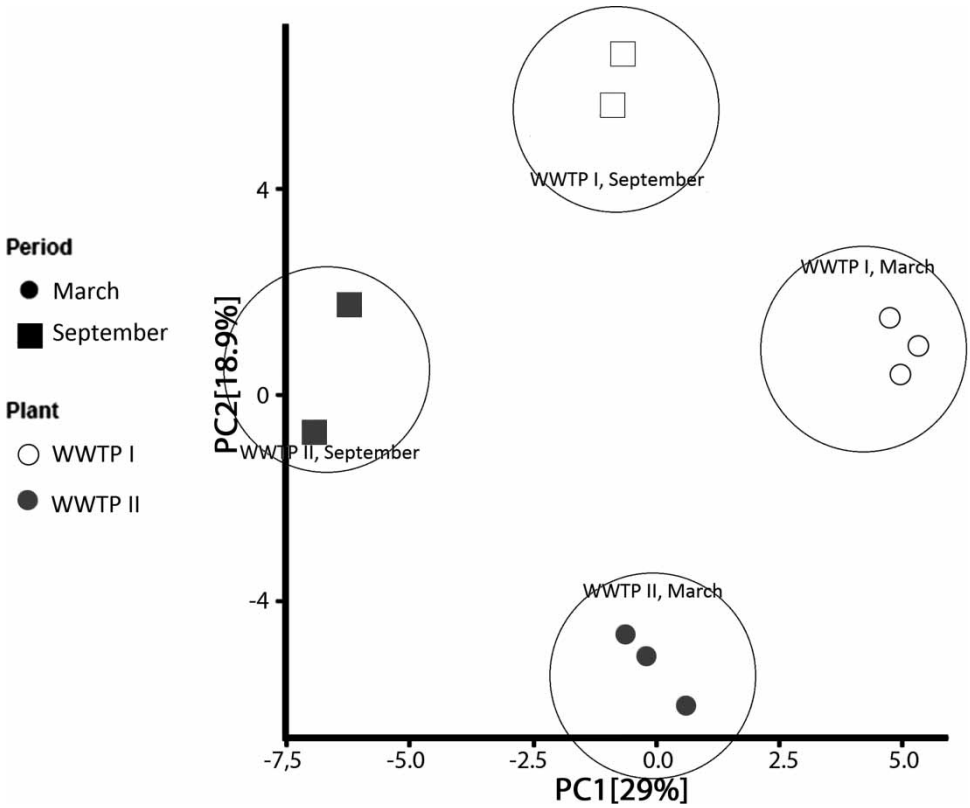

Figure 3 | PCA presenting distinction in the microbial communities in Polish WWTPs by sampling period. Variables plotted onto the two-factor plane.

higher left quarter. Samples from WWTP I are coloured white and are placed on the higher right quarter, while samples from WWTP II are located on the lower left quarter. This is a larger effect of sampling than was normally observed in Danish plants. Four groups of samples, which are close to each other and have similar microbial composition, can be easily distinguished in Figure 3. However, two principal components from PCA presented below explained less than 50% of variance.

PCA of the microbial communities in Polish and Danish samples (Figure 4) indicated differences between Danish WWTPs treating domestic, industrial and mixed wastewater, whereas microbial communities in Polish WWTPs were clustered together and were similar to each other. Samples from WWTP I (red points, upper encircled points in black & white (B&W) version of paper) were located among Danish domestic WWTP samples (green points, grey points in B&W version), whereas samples from WWTP II (blue points, lower encircled points to the right in B&W version) were somewhere between Danish domestic (green points, grey points in B&W version) and mixed (violet points, light grey points in the bottom part

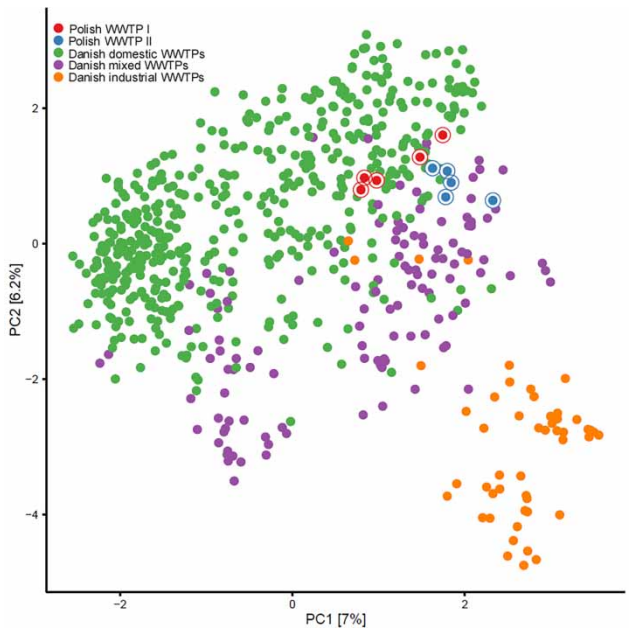

Figure 4 | PCA presenting microbial communities in samples from Polish WWTPs among Danish samples. Variables plotted onto the two-factor plane. The full colour version of this figure is available in the online version of this paper, at http://dx.doi.org/10.2166/wst.2017.015.

of B&W version) WWTPs. It should be stated that WWTP I treated typical domestic wastewater, while in WWTP II 30–50% of influent came from industry. These data explain only small parts of total variation (two principal components explained less than 13.2% of variance) and should be treated with caution.

DISCUSSION

Studies on the AS from Danish WWTPs gave a large contribution to understanding the interactions between the many organisms present in AS. Bacteria were investigated in enrichment cultures or *in situ* using methods such as FISH and microautoradiography to test their ecophysiology and individual substrate specificities (Mielczarek *et al.* 2012, 2013). Conceptual models of microbial communities involved in nutrient removal from wastewater were developed (Nielsen *et al.* 2010, 2012). Whereas more and more researchers from different countries used 16S ribosomal RNA (rRNA) amplicon sequencing and reported the same taxa in WWTPs, Poland lacked similar data for comparison. The Microbial Database for Activated Sludge (MiDAS) field guide (McIlroy *et al.* 2015) was used in this study to connect the identity of abundant and process-critical microorganisms in Polish WWTPs to available data for bacterial functional importance.

Microbial communities in the tested samples were rather similar and relatively stable over the whole period of the study; the specific genera found in the studied Polish WWTPs are also common in Danish plants (Figure 1). However, three of the most dominant genera in Danish WWTPs, i.e. *Tetrasphaera* (engaged in biological phosphorus removal), *Trichococcus* (heterotrophic bacteria) and *Microthrix* (filamentous heterotrophic bacteria) were only found in very low abundance in the examined Polish WWTPs. On the other hand, in Polish WWTPs *Proteobacteria* (involved in chemical oxygen demand (COD) and nitrogen removal) were very abundant, as well as genera of phylum *Bacteroidetes*, which in general are not observed at such a level in Danish WWTPs.

The most abundant 30 bacterial groups in Polish WWTPs constituted more than half of the AS biocoenosis and generally belonged to the core community described by Saunders *et al.* (2016), where 63 OTUs constituted up to 68% of the total reads. However, Polish WWTPs had a less diverse microbial community, and the dominant genera were more abundant than in Danish WWTPs, e.g. heterotrophic *Rhodoferax* accounted for up to 20%.

The present study confirmed the hypothesis of Saunders *et al.* (2016) that regionally abundant populations contribute to core communities. Neutral community assembly can play a role in modelling the diversity of functional guilds in AS, as was the case for ammonia-oxidizing bacteria in WWTPs (Ofiteru *et al.* 2010). Although most of the 30 abundant bacteria in the Polish WWTPs were part of the core, the ones outside it can be interesting and also important.

The abundance of *Zoogloea*, denitrifiers involved in production of amyloid adhesins in the extracellular polymeric substance and important for floc formation, may determine the structure of flocs. In the tested WWTPs, which experienced operational problems connected with sludge bulking, the relatively high abundance of SBR1029 (12th position in ranking) may be worth investigating. These members of *Chloroflexi* could be responsible for impairing the settling properties of AS flocs, while AS foaming could be caused by *Arcobacter* with a potentially high cell hydrophobicity. Guo *et al.* (2015) showed that these bacteria, together with *Gordonia*, *Mycobacterium*, *Clostridium* XI, *Simplicispira*, *Flavobacterium* and *Williamsia*, were consistently more frequent in the foam than in the AS. Moreover, 'comammox' (*Nitrospira*) and NOBs (*Candidatus* Nitrotoga) constituted bigger fractions in Polish than in Danish WWTPs and probably should be included in the core for Poland. Alawi *et al.* (2009) showed that *Candidatus* Nitrotoga can be numerically abundant in bacterial communities adapted to lower temperatures. Results of the present study, showing significant abundance of these NOBs in Polish WWTPs, can be successfully used to investigate the dominance of various groups of nitrite oxidizers, taking into consideration attempts to find factors selecting for NOBs (Lücker *et al.* 2014; Saunders *et al.* 2016). On the other hand, *Ferribacterium* was alleged by Ziembińska *et al.* (2009) to oxidize ammonia in anaerobic niches of AS in the aerated bioreactor. However, there is still no research confirming or excluding the nitrification abilities of *Ferribacterium*.

Seasonal variations have been observed in the abundance of bacterial genera. PCA explaining a small part of total variation (Figure 3) confirmed that microbial communities in the AS of Polish WWTPs was clearly distinguished by sampling period (even with a gap of up to 3 years between the samples; on the other hand it should be highlighted that two factors in obtained PCA explained 47.9% of variance), and they can be easily classified to their plant of origin. However, Student's *t*-test showed significant differences between the data sets from two WWTPs only in the case of the abundance of heterotrophic

Ferruginibacter (*Bacteroidetes*). As far as these results are interesting, they need to be confirmed in a study with more frequent sampling to see how this changes the results of PCA and what causes that effect.

Surveys covering more Polish WWTPs would help to identify the most significant genera and to distinguish the Polish core microbial community. This is an interesting aspect, because only 30 of the most abundant bacterial groups constituted on average more than a half of the AS biocoenosis, and apart from heterotrophic bacteria they also included 'comammox' and NOBs (*Nitrospira* and *Candidatus* Nitrotoga, respectively) as well as a potential group responsible for AS bulking (*Chloroflexi* SBR1029). Comparison of a microbial population structure in an examined WWTP to the well-defined core community in Polish WWTPs could be a powerful tool, not only describing the current condition of AS communities in WWTPs, but maybe also predicting operational problems (like bulking). In the future, it could also give possibilities of implementing quick counteractions to such problems.

CONCLUSIONS

Bacterial communities in Polish and Danish WWTPs are similar to each other. Nevertheless, Polish WWTPs show a higher abundance of bacteria involved in nitrogen and COD removal (*Proteobacteria* and *Bacteroidetes*), while polyphosphate accumulating bacteria are the dominant bacterial group in Danish plants. Heterotrophic *Rhodoferax* were the most dominant bacteria in Polish WWTPs and accounted for up to 20% of pairs of reads. The microbial community structures in the examined Polish WWTPs were relatively similar to each other, but showed strong seasonal variations which are not normally observed in Danish WWTPs. Confirmation of these seasonal variations, however, requires additional testing at a higher sampling frequency.

The most abundant 30 bacteria constituted more than half of the AS community in the Polish WWTPs and mostly belonged to the core community described by Saunders *et al.* (2016). However, the microbial diversity in Polish WWTPs is lower, while the dominant genera are more abundant than in Danish WWTPs.

ACKNOWLEDGEMENTS

The study was financially supported by the Faculty of Building Services, Hydro and Environmental Engineering of Warsaw University of Technology (grant number 540M/1110/0022/000). We would like to thank: DNASense for their magnificent report and sharing data about Danish WWTPs; Foundation for Polish Science for supporting an inspiring internship at Department of Chemistry and Bioscience – Section for Biotechnology (Aalborg University). If you are interested in obtaining metagenomics data from the study, please contact the corresponding author or download them using the link https://www.dropbox.com/sh/soeyh1u38825o2v/AAD-NJKsUonf4QM8XyzEb0Tia?dl=0.

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First received 28 July 2016; accepted in revised form 16 December 2016. Available online 2 January 2017