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Impact of Operational Parameters on Bacterial Community in a Full-Scale Municipal Wastewater Treatment Plant

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Abstract

A bacterial community in activated sludge from a full-scale municipal wastewater treatment plant was monitored throughout the year with the use of FISH, RISA and DGGE techniques. In the investigated range of temperatures (11.9–21.6°C), a rise in temperature resulted in a lower total bacteria richness, while organic load rate changes from 0.09 to 0.21 g COD·g $TSS^{-1}\cdot d^{-1}$ were positively correlated with the number of bands in RISA patterns. The most diverse pattern (29 different bands) was characteristic for the activated sludge sample collected at the end of January at wastewater temperature of 11.9°C. The ammonia-oxidising bacteria community did not change during the study, and comprised of 4 different bacterial populations with one dominant species closely related to *Nitrosospira* sp. REGAU (GenBank accession number AY635572.1). The percentage of ammonia-oxidising bacteria in the activated sludge varied from 6.2 to 19.5% and depended on temperature ($R=0.61$, $p=0.02$) and organic load rate ($R=-0.55$, $p=0.04$).

Key words: ammonia-oxidising bacteria, DGGE, FISH, molecular analysis of activated sludge, RISA

Introduction

One of the significant aspects of microbial community investigations is the evaluation of the impact of environmental conditions on bacterial consortia. In engineered systems, *e.g.* wastewater treatment plants there is a possibility to manipulate the technological parameters of the process and as a result to influence the microbial communities involved in removal of pollutants. It has recently been suggested that the diversity of specific bacterial groups in activated sludge influences the functioning of the reactor (Daims *et al.*, 2001). The presence of many microorganisms able to conduct a specific process increases the probability that a change of environmental conditions does not worsen the effectiveness of wastewater treatment, since one of the species will adapt and maintain the specific metabolic pathway (LaPara *et al.*, 2002). Taking this into consideration, the operational parameters of the wastewater treatment plant should be selected to favour the development of a highly diversified bacterial community.

Ammonia-oxidising bacteria (AOB) are of universal importance in activated sludge community since ammonia oxidation is the limiting step for nitrogen removal from wastewater. The growth rate of AOB is slow and their population activity may be affected by operational/environmental factors *e.g.* solids retention time, ammonium and organic carbon concentration in the influent, pH or temperature (Nogueira *et al.*, 2002; Avrahami *et al.*, 2003; Kuo *et al.*, 2006). Research by Siripong and Rittmann (2007) showed that monitoring of changes in the nitrifiers community can assist plant operators in preventing nitrification failure and the washing out of these bacteria from the system. It is therefore important to evaluate an impact of the operational parameters of the wastewater treatment process on AOB community in activated sludge.

The use of molecular techniques allows an insight to be gained into changes in bacterial communities in activated sludge in relation to environmental conditions. Much recent research focuses on investigating bacteria involved in wastewater pollutants removal (Onuki *et al.*, 2000; Ariesyady *et al.*, 2007; Zhao *et al.*, 2008) but information about the relation between bacteria diversity and operating parameters is still limited. Juretschko *et al.* (2002) showed that consortia of microorganisms are influenced by wastewater composition and that diversity of bacteria is higher in systems treating municipal wastewater in comparison to industrial sewage treatment plants. Rowan *et al.* (2003) revealed that the bacterial diversity is linked to the type of bioreactor. The research indicated a higher diversity of

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AOB in the trickling filter than in the biological aerated filter (BAF), and that the performance of the trickling filter was better than the BAF. Cydzik-Kwiatkowska and Wojnowska-Baryła (2008) proved that a higher AOB diversity was observed when only carbonates were present in the wastewater in comparison to conditions in which sodium acetate was introduced to the reactors and that a lower ammonia nitrogen load favoured a more diverse AOB community.

In the present study we focused on determining the impact of technological parameters on ammonia-oxidising and total bacteria community in activated sludge from a full-scale municipal wastewater treatment plant operating in temperate climate. In our research we combined fluorescent *in situ* hybridisation (FISH), Denaturing Gradient Gel Electrophoresis (DGGE), Ribosomal Intergenic Spacer Analysis (RISA) and DNA sequencing in order to obtain comprehensive results.

Experimental

Materials and Methods

Description of wastewater treatment plant. A one-year study was conducted on a full-scale activated sludge wastewater treatment plant (WWTP) located in Poland (temperate climate). The WWTP treats on average 30,000 m^3 of municipal wastewater per day and discharges it to the Łyna River. The treatment unit of the plant involves grates, grit chambers, primary clarifiers, an anaerobic chamber, predenitrification chamber, aeration chambers for simultaneous nitrification and denitrification (16,380 m^3), and secondary clarifiers.

Activated sludge sampling. 14 activated sludge samples were collected at different dates from the WWTP. Three samples were collected in autumn: 1 – 14.10.2005, 2 – 8.11.2005, and 3 – 5.12.2005, three in winter: 4 – 03.01.2006, 5 – 31.01.2006, and 6 – 27.02.2006, four in spring: 7 – 27.03.2006, 8 – 24.04.2006, 9 – 10.05.2006, and 10 – 01.06.2006, and four in summer: 11 – 27.06.2006, 12 – 26.07.2006, 13 – 4.09.2006, 14 – 20.09.2006. Samples were collected directly from the aeration tank, transported on ice to the laboratory (< 30 min) and processed immediately.

Analytical methods. Complete operating results were delivered by the WWTP laboratory, which monitors the activated sludge process daily. Analyses were performed according to Polish Standards (www.pkn.pl). Computations of technological parameters such as sludge retention time (SRT) or nitrogen use for biomass synthesis were performed as described in Metcalf and Eddy (1991).

Molecular analyses of activated sludge samples. Genomic DNA was isolated in duplicate from 400 mg of centrifuged sludge sample using FastDNA® SPIN®Kit (Q-BIOgene), according to the manufacturer's protocol. Quality and quantity of isolated DNA was measured spectrophotometrically using Biophotometer (Eppendorf). The DNA extracted from a sample was mixed and frozen at –20°C for further analyses.

All PCR reactions were performed in Eppendorf Mastercycler Gradient (Eppendorf). The bacterial RIS (Ribosomal Intergenic Spacer) was amplified with primers *1* and *2* (Table I). Amplified fragments contained RIS plus approximately 380 bp corresponding to flanking regions of genes coding for 16S and 23S rRNA. The PCR mixture contained 50 ng of extracted total DNA, 0.5 µM of each primer, 100 µM of deoxynucleoside triphosphate mixture (Promega), 1.5 U of GoTaq® DNA Polymerase (Promega), 6 µl of reaction buffer supplied with polymerase, 1.5 mM $MgCl_2$ and sterile water to a final volume of 30 µl. The PCR amplification was carried out using the following program: 95°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 43°C for 45 s, extension at 72°C for 1 min, and a final elongation at 72°C for 5 min. The presence of PCR products was confirmed by analysing 5 µl of the product on a 0.8% agarose gel stained with ethidium bromide.

After successful DNA amplification, 5 µl of PCR products were applied to 6% polyacrylamide gel (29:1 acrylamide:bisacrylamide). Electrophoresis was carried out at 60 V in 1x TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA; pH 8.0). In order to avoid overlapping of bands and fully exploit the microbial diversity, each sample was electrophoresed for 60, 120 and 180 min and all photos obtained were used for further analysis. After electrophoresis, the gel was

Table I
PCR primers used in the study

Primer	Sequence (5'-3')	Annealing temperature	Target sequence	Source
amoA-2R *amoA-1F*	ccc ctc tgc aaa gcc ttc ttc [a]ttt cta ctg gtg gt	60°C	*amoA*	Rotthauwe *et al.* (1997) (modified by Nicolaisen and Ramsing, 2002)
1 *2*	ttg tac aca ccg ccc gtc a gt act tag atg ttt cag ttc	43°C	*RIS*	Dolzani *et al.* (1995)

[a] – cgc cgc gcg gcg ggc ggg gcg ggg gcg ggg

Table II
Probes used for hybridisation

Probe	Sequence (5'-3')	Label	% formamide	Specificity
EUB338	GCTGCCTCCCGTAGGAGT	FITC	20	Eubacteria
Nso190	CGATCCCCTGCTTTTCTCC	Cy3	55	Majority of AOB: *Nitrosomonas*, *Nitrosococcus*, *Nitrosolobus, Nitrosovibrio*, *Nitrosospira*

stained with SYBRgold (Molecular Probes) at 10,000x dilutions in 1x TBE buffer for 30 min, viewed with an ultraviolet transilluminator and recorded with CCD camera (Gel Logic 200, Eastman Kodak Company). Bands were detected automatically from digital images of the gel using KODAK 1D 3.6 Image Analysis Software (Eastman Kodak Company). The size of PCR products was estimated using 100 bp O'GeneRuler ladder (Fermentas).

Amplification of *amoA* gene fragment for DGGE was performed as in RIS reactions, except for applied primers and annealing temperature (Table I). DGGE electrophoresis was performed with a D-CODE Universal Mutation System (Bio-Rad). The PCR samples were applied to 8% polyacrylamide gel (37.5:1 acrylamide:bisacrylamide) in a 0.5 × TAE buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA; pH 7.5) with a denaturing gradient ranging from 30 to 70%. Denaturation of 100% corresponded to 7 M urea and 40% formamide. Electrophoresis was run at a constant voltage of 80 V at 60°C. After electrophoresis, the gel was processed as described above. The most intense band was cut from the gel, reamplified using specific primers (amoA-1F with no GC clamp) and sequenced by a specialist laboratory (www.oligo.ibb.waw.pl). The nucleotide sequence was compared with sequences in the GenBank using the BLASTn program (Altschul *et al.*, 1997) and deposited in the GenBank under accession no. GU073249).

The biomass used for FISH analysis was fixed according to Amann *et al.* (1990). The prepared sample was vortexed and kept at 4°C for 1 day. After centrifugation (3 min, 4,000 rpm), the supernatant was removed and the sample was resuspended in a mixture of 1x PBS and 96% ethanol (volume ratio of mixture components 1:1) and stored at a temperature of –20°C. For analysis, 10 µl of sample was spread on each well of the glass slides (Marienfeld Laboratory Glassware), dried for about 10 min at 46°C, and dehydrated by serial immersions of the slide in 50, 80, and 96% (v/v) ethanol (3 min each). The composition of the hybridisation buffer was the following: 180 µl of 5 M NaCl, 20 µl of 1 M TrisHCl (pH 8.0), 550 µl of formamide, 250 µl of ultra clean water and 2 µl of 10% (w/v) SDS. The washing buffer, containing 100 µl of 5 M NaCl, 1000 µl of 1 M TrisHCl (pH 8.0), 500 µl of 0,5 M EDTA (pH 8.0), 50 µl of 10% (w/v) SDS, and distilled water to complete the buffer to 50 ml, was preheated in a water bath to 48°C. Two molecular probes, as proposed by Mobarry *et al.* (1996) were used for hybridisation (Table II). In order to detect Eubacteria, only one molecular probe EUB338 was used (Chae *et al.*, 2008) that would make it simpler to utilize the FISH method in wastewater treatment plants.

A mixture of 140 µl of hybridisation buffer and molecular probes (10 pmol/µl) was prepared and placed in each well. The slide was transferred into the hybridisation chamber and incubated at 46°C for 3 h. After hybridisation the slide was incubated in the washing buffer for 10 min in a preheated water bath at 48°C. The slide was rinsed with cold distilled water and dried. Slides were mounted in VectaShield (Vector Laboratories) embedding medium. Epifluorescence microscopy by Nikon Eclipse (Nikon, 100x objective) was used for examination. For a reproducible and statistically correct result, 30 images for each probe taken from 30 different positions of the slide were analysed (Hall *et al.*, 2003).

The area covered by specific probe Nso190 and the area covered by the EUB338 probe in the same field of the slide glass, taken as images, were calculated by using ImageJ software (National Institutes of Health, USA). The occupation ratio of the area given by Nso190 to the area from EUB338 gives a direct measurement of the number of active ammonia-oxidising bacteria (Rittmann *et al.*, 1999).

Statistical analysis. The relationships between bacterial richness and chemical parameters in the effluent of the treatment facility were determined by correlation analyses using the STATISTICA 7.0 programme (StatSoft, USA). The strength of the correlation was evaluated according to Stanisz (2000). Analyses were carried out at a confidence interval of 95%. In the text, after ± the standard deviation is given. On the basis of RISA patterns, distance matrix analyses were performed according to the method of Nei Li (Nei and Li, 1979), using the DGGEstat 1.0 programme (van Hannen, the Netherlands Institute for Ecological Research, NIOO-KNAW, the Netherlands). Binary sequences were generated by determining the number and position of bands in individual lanes compared to all of the band positions detected in all of the lanes. The samples were clustered using the unweighted pair group method of arithmetic averages (UPGMA), bootstrapping was conducted with 1000 replicates.

Results and Discussion

In a temperate climate one of the major factors influencing biological wastewater treatment efficiency is temperature. In this study the temperature of wastewater fluctuated considerably throughout the year. The maximum temperature amplitude of wastewater during the study period was 9.7°C (Fig. 1a).

The operating data for the facility are presented in Table III. On the base of organic (B_C) and nitrogen (B_N) load rates it can be concluded that the plant represented a system with a low load rate. The average concentration of organic compounds expressed as COD in the influent was 416.1 ± 66.1 mg COD/l, while in the effluent during the year it averaged 37.0 ± 5.1 mg COD/l (Fig. 1b). COD removal maintained at the level of 90.8 ± 2.5%. The average concentration of total nitrogen (TN) in the influent was 62.1 ± 8.9 mg N/l. The highest TN concentration in the effluent of 20.8 and 16.1 mg N/l was observed at the turn of winter and spring (Fig. 1c). During the rest of the year this value did not exceed 13 mg N/l. A high percentage of total nitrogen removal (84.1 ± 6.4%) provided evidence of nitrification. It was calculated that 6.4 ± 2.9 mg N/l was used for biomass synthesis. Regarding phosphorus concentration, in the influent it averaged 10.6 ± 3.0 mg P/l, while in the effluent it was in the range of 0.3–1.7 mg P/l, except the sample for January, when the concentration of phosphorus increased to 4.1 mg P/l (Fig. 1d). The average efficiency of phosphorus removal in the system was 89.4 ± 8.9%.

An identification of microorganisms involved in pollutants removal gives us some information about properties of activated sludge, however, the complexity of factors influencing activated sludge in WWTP results in extremely variable microbial structure that is unlikely to occur in another WWTP. It is therefore more important to reveal general dependences between the microbial community as a whole and technological parameters of the process. In our research the bacterial

Table III
Summary wastewater treatment plant operating data during the study period.

Parameter	Mean value ± standard deviation
Sludge volume index (ml/g)	260.0 ± 77.0
Total suspended solids (mg TSS/l)	5229.0 ± 1117.2
Organic loading (g COD·g TSS^{-1}·d^{-1})	0.15 ± 0.03
Nitrogen loading (g N·g TSS^{-1}·d^{-1})	0.02 ± 0.005
COD/TKN (g COD/g N)	6.8 ± 1.3
Sludge yield (g TSS/g COD)	0.3 ± 0.1
HRT (h)	13.0
SRT (d)	21.6 ± 14.9

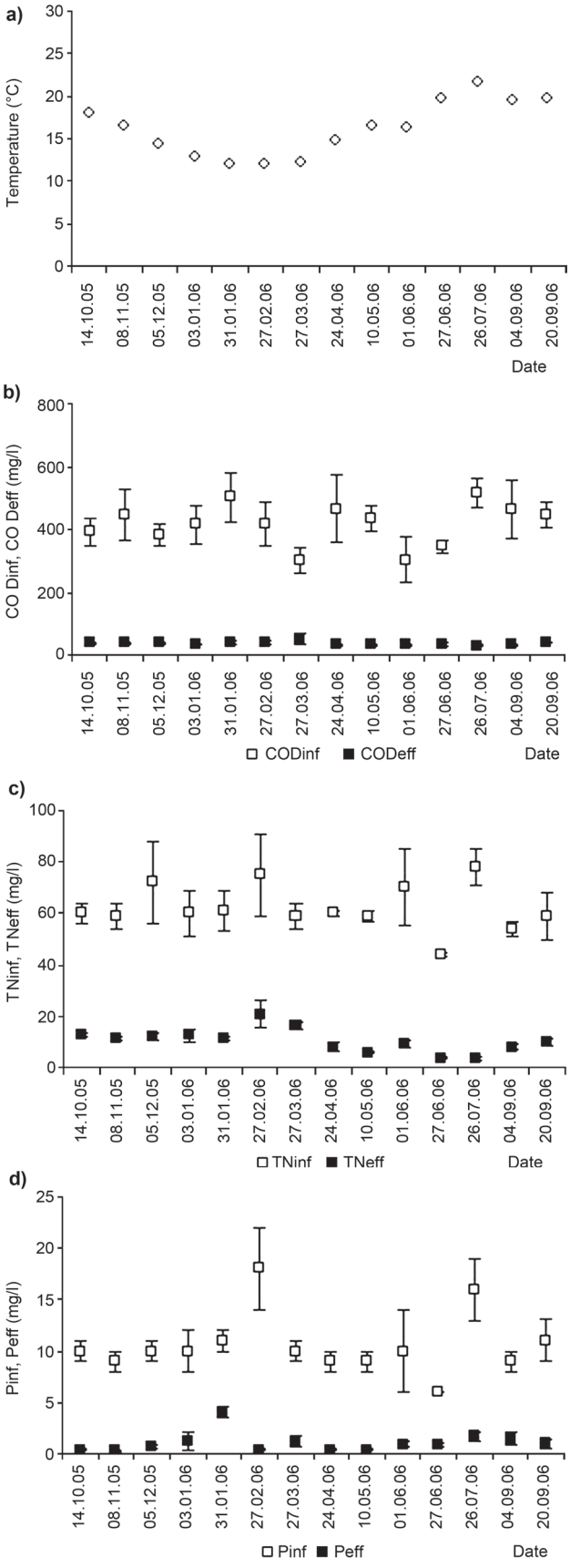

Fig. 1. Temperature (a) and influent ($_{inf}$) and effluent ($_{eff}$) quality of wastewater: COD (b), total nitrogen (c), phosphorus (d).
Each point is the mean of 4 measurements, vertical bars indicate standard deviation.

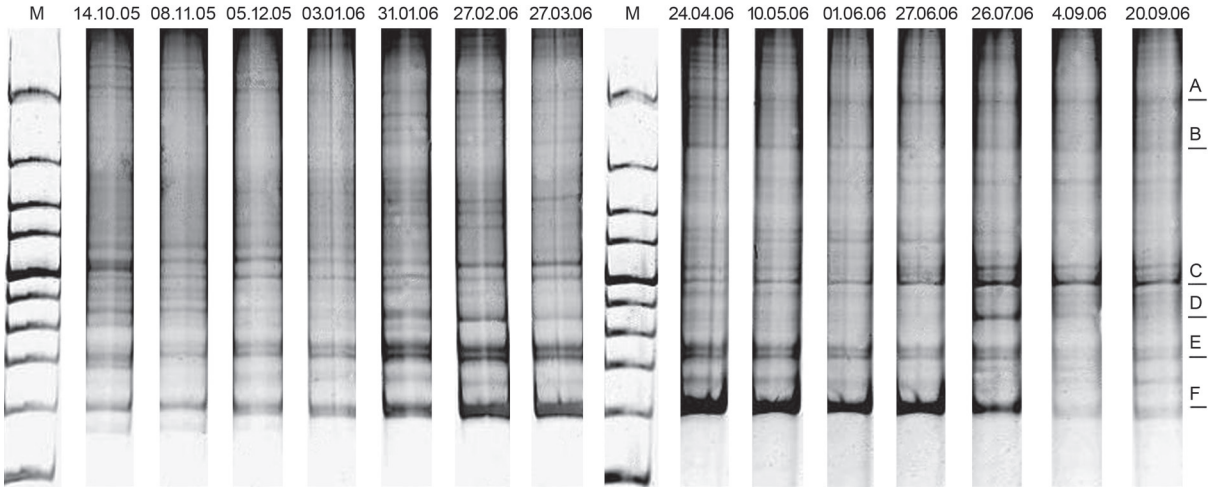

Fig. 2. RISA fingerprints of bacterial community from activated sludge.
Lanes are labelled with the dates of sampling.
Exemplary bands are marked with capital letters, M – molecular weight marker 100 kb O'GeneRuler (Fermentas).

community changed moderately throughout the year while the effluent quality remained relatively stable. The RISA fingerprints were complex, and the mean number of bands per lane was 22.1 ± 4.2 (Fig. 2). Several bands were present only temporarily (*e.g.* bands labelled B and D), while some bands were detectable throughout the whole year, though their intensity varied sometimes with time (*e.g.* bands A, C, E, F). The intensity of band F was significantly higher during the period from March to June, suggesting that this species was present in the highest number in spring. It is probable that the bands detected in all the samples are the crucial bacterial populations for activated sludge performance. The richness of bacterial consortia expressed by the number of different bands in the RISA pattern fluctuated during the year (Fig. 3). The most diverse patterns were characteristic for activated sludge samples collected at the end of the winter and at the beginning of the spring. The highest number of bands (29) was noted in the sample collected 31.01.2006.

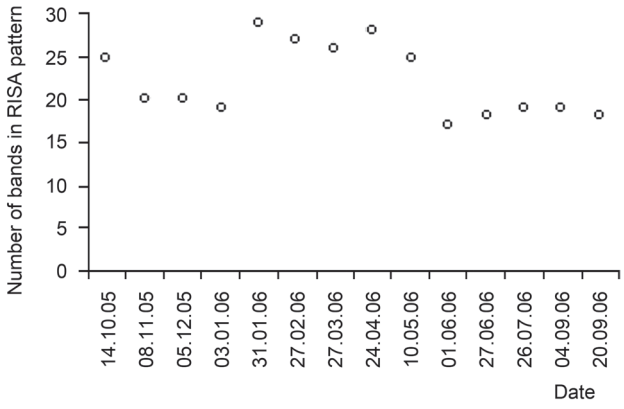

Fig. 3. The number of bands in RISA patterns obtained for activated sludge samples collected at given dates.
Grey vertical lines separate samples from different seasons of the year.

The higher temperature of wastewater leads to an increase in the population density and metabolic activity, and shortens the generation time of bacteria in activated sludge (Saleem *et al.*, 2003). Our research showed that a statistically significant correlation occurred between bacterial richness of sample and the temperature of wastewater (Fig. 4a). The value of Pearson's correlation rank (R = – 0.61) indicates that the negative correlation between the analysed parameters was strong. Moreover, richness of microorganisms was correlated with organic load rate (B_C) of the system (Fig. 4b). During the year of sampling B_C varied from 0.09 to 0.21 g COD · g $TSS^{-1} \cdot d^{-1}$ and bacteria richness increased with increasing B_C. Similar results were obtained by Xia *et al.* (2008). Authors investigated the microbial community structure response to different ratios of carbon to nitrogen (C/N 3:1, 5:1, 10:1) in wastewater in a suspended carrier biofilm reactor with simultaneous nitrification and denitrification. On the basis of DGGE and FISH analyses it was shown that total diversity of microbial community structure increased with increasing organic carbon concentration in wastewater. It is worth noting that the correlation obtained in our study is probably true only in a limited range of organic load rate. At a very high B_C, the diversity tends to decrease because a large quantity of organic carbon in wastewater stimulates dynamically growing bacteria with a very short generation time and these compete out other species and dominate the bacterial population.

Cluster analysis of RISA fingerprints revealed that the bacterial community changed gradually with the passage of time (Fig. 5). Three distinct branches were formed in the dendrogram. The first four samples formed the first branch, the following five the second branch which was connected to the third branch clasping the last five samples. The bootstrap values for the

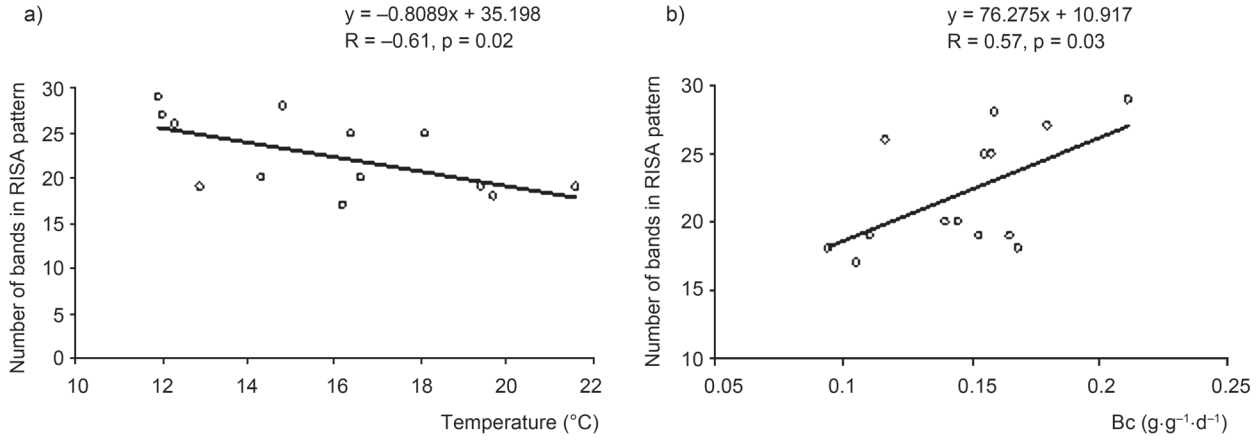

Fig. 4. Relationship between the number of bands in RISA pattern and a) wastewater temperature, b) organic load rate during the study

third branch was 90.7, suggesting that summer bacterial communities were the most similar to each other, and differed significantly from consortia developed in other seasons of the year. LaPara and Ghosh (2006) noted a similar situation in research carried out in a full-scale municipal wastewater treatment facility. DGGE fingerprints obtained on the basis of activated sludge samples from 25th June to 3rd September formed a distinct branch in the dendrogram.

DGGE of PCR-amplified *amoA* gene fragments revealed that there were 4 different AOB populations

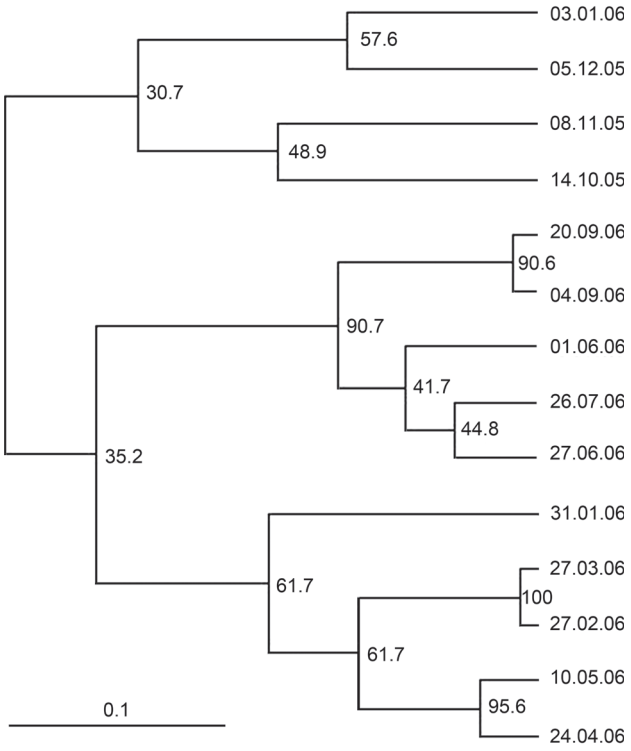

Fig. 5. Dendrogram revealing the relatedness of RISA fingerprints of the bacterial community from aeration tank of investigated wastewater treatment plant.

Branches are labelled with the dates of sampling; bootstrap values for branches are given at the nodes.

per sample, and that the ammonia-oxidising bacteria community did not change throughout the study (data not shown). The coexistence of various nitrifiers with different growth and survival characteristics may be valuable for maintaining the stability and performance of nitrifying bioreactors. Sequencing of the dominant band indicated that the partial *amoA* gene sequence was similar (89% similarity) to the sequence of ammonia monooxygenase from *Nitrosospira* sp. REGAU (GenBank accession number AY635572.1). LaPara and Ghosh (2006) investigated the AOB community in a full-scale municipal wastewater treatment facility using the nested PCR-DGGE technique. The fingerprint obtained was also dominated by one band. This band was, however, not phylogenetically related to any known AOB. Siripong and Rittman (2007), using the T-RFLP technique, examined the impact of temperature, sludge age, input of industrial wastewater and other technological parameters on the diversity of nitrifiers in seven water reclamation plants in two different seasons (winter and summer). Authors have shown that only the seasonal temperature variations changed the nitrifying community, in particular the balance between *Nitrosospira* and *Nitrosomonas*, however, both *Nitrosospira* and *Nitrosomonas* coexisted in winter and summer samples. Authors' conclusion is that a combination of low temperature and high SRT may favour *Nitrosospira*. Limpiyakorn *et al.* (2006) investigated AOB communities in 12 full-scale sewage activated sludge systems in three different seasons using PCR-DGGE-cloning-sequencing of 16S rRNA genes. However, in this case in all samples one of the identified sequence types of the *Nitrosomonas oligotropha* cluster was the dominant AOB in every system and every season studied.

FISH analyses revealed that the percentage of ammonia-oxidising bacteria in activated sludge decreased from 19.5 to 14.2% in autumn (Fig. 6), and to 6.2–9.7% in winter. In spring, the percentage of AOB increased to 14.9% and remained at a similar level until the end

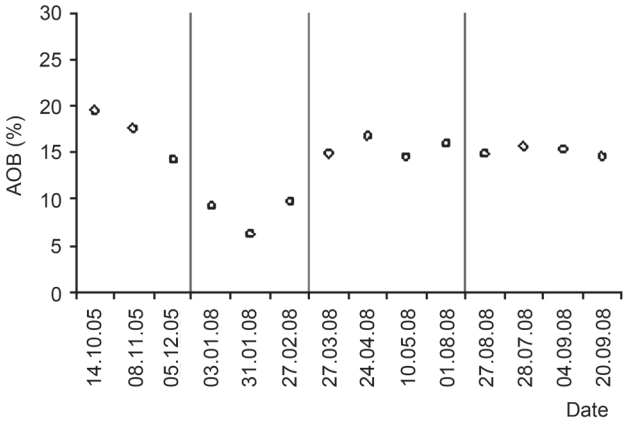

Fig. 6. Percentage of AOB in activated sludge samples collected at given dates.
Grey vertical lines separate samples from different seasons of the year.

of summer. The values obtained are consistent with the observations of Okabe *et al.* (1999) who reported that ammonia-oxidising bacteria comprise from 5 to 20% of bacteria in domestic wastewater systems. We showed that there was a significant correlation ($R = 0.61$, $p = 0.02$) between the wastewater temperature and percentage of AOB in activated sludge (Fig. 7a). The temperature dropped below 12°C in February which caused a decrease in AOB participation in the activated sludge to 6.2%. Low temperatures generally have a drastic effect on bacterial process rates and this is especially important when nitrification processes are concerned: below 15°C, the nitrification rate drops sharply, and is reduced by 50% at 12°C (Focht and Verstraete, 1977). However, Rittmann and Snoeyink (1984) mentioned that nitrification processes have the potential to achieve a good ammonia removal even at low temperatures. The critical aspect seems to be the maintenance of slow-growing nitrifying bacteria in the system. In our research, SRT applied at the level of 21.6 ± 14.9 d guaranteed that these essential bacteria would not be washed out from the wastewater treatment plant.

Throughout all the seasons studied the activated sludge process showed evidence of stable nitrification with efficiency at the level of 73.3–91.4%. No relationship between nitrification efficiency and ammonia-oxidising bacteria contribution in activated sludge ($R = 0.06$, $p = 0.83$) was noted. This result is not consistent with previous studies conducted in full-scale nitrifying trickling filters, suggesting that the concentration of AOB has an impact on effluent quality (Dionisi *et al.*, 2002). In the research of Ebie *et al.* (2002), the FISH method with 16S rRNA-targeted oligonucleotide probes was used for quantitative estimation of ammonia-oxidising bacteria in an anaerobic-aerobic activated sludge process with membrane filtration. At the beginning of the experiment, the occupation ratio of AOB increased as nitrification progressed. Later, similarly to our results, the occupation ratio decreased, but good nitrification ability was, however, preserved.

The fact that organic carbon influences AOB populations in wastewater treatment systems is discussed in the literature (Lazarova *et al.*, 1998; Nogueira *et al.*, 2002). Organic carbon compounds in wastewater stimulate heterotrophic bacteria which compete for oxygen with nitrifiers and may produce intermediary metabolic byproducts, which inhibit AOB (Gieseke *et al.*, 2001). Xia *et al.* (2008) showed that the population of nitrifiers was inversely proportional to the C/N ratio in wastewater with an average fraction of AOB and nitrite-oxidising bacteria (NOB) to all bacteria of 5.4, 4.8, 3.1% and 4.6, 3.5, 2.7%, respectively, as the C/N ratio changed from 3:1, 5:1 to 10:1. Our research was conducted in a full-scale municipal wastewater treatment plant with a low organic load rate at a level of 0.15 ± 0.03 g COD × g $TSS^{-1} \times d^{-1}$. Even relatively low organic load rate, however, influenced the AOB population in activated sludge (Fig. 7b; $R = -0.55$, $p = 0.04$). In practice, the organic load rate in a wastewater treatment plant can be easily adjusted. On the basis of our research it may be concluded that the negative impact

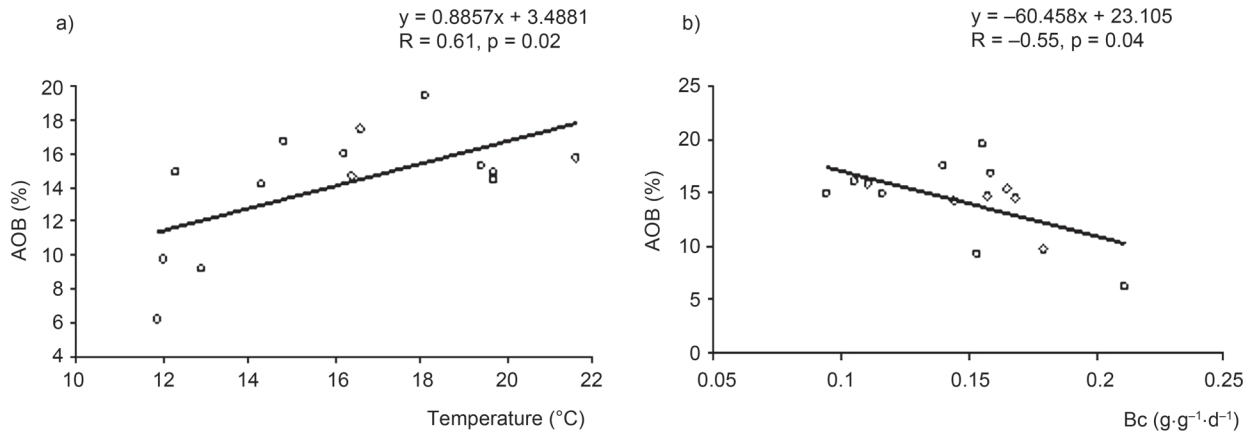

Fig. 7. Relationship between the participation of AOB in activated sludge and a) wastewater temperature, b) organic load rate

of low temperatures on AOB participation in activated sludge can be minimized by lowering the B_C value.

Conclusions. Presented research possesses utilitary values. It allows observing general trends in microorganisms' consortia during the year of plant operation in temperate climate, and characterizing the bacterial community, especially AOB, in activated sludge, in correlation with operational parameters of a full scale WWTP. The following conclusions may be drawn from the research:

1. The total bacterial community in activated sludge changed moderately with the passage of time. Bacterial communities from summer samples were the most similar to each other and differed significantly from consortia developed in other parts of the year.
2. In investigated range of values the total bacteria richness was correlated with the two parameters of wastewater treatment process that is the temperature ($R = -0.61$, $p = 0.02$) and organic load rate ($R = 0.57$, $p = 0.03$).
3. The percentage of AOB in activated sludge varied from 6.2 to 19.5% during the year of study and depended on temperature ($R = 0.61$, $p = 0.02$) and organic load rate ($R = -0.55$, $p = 0.04$).
4. The AOB community comprised of 4 different bacterial populations and did not change during the investigation period, with one dominant species closely related to *Nitrosospira sp.* REGAU.

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