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Understanding the efficacy of influent waste water on microbial community structure of activated sludge process

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The assembling of microbial consortia in wastewater treatment facilities is a significance of environmental conditions. In the present research work, activated sludge from different wastewater treatment plants (WWTPs) were exploited at a molecular level to determine the influence of the complexity of the influent composition on the species structure and the diversity of bacterial consortia. The community fingerprints and technological data were subjected to the canonical correspondence and correlation analyses. The number of separated biological processes realized in the treatment line and the presence of industrial wastewater in the influent were the key factors determining the species structure of total and ammonia-oxidizing bacteria in biomass. The N_2O-reducers community composition depended significantly on the design of the facility; the highest species richness of denitrifiers was noted in the WWTPs with separated denitrification tanks. The contribution of industrial streams to the inflow affected the diversity of total and denitrifying bacterial consortia and diminished the diversity of ammonia oxidizers. The obtained data are valuable for engineers since they revealed the main factors, including the design of wastewater treatment plant, influencing the microbial groups critical for the stability of purification processes.

Key words: Denitrification, waste water treatment plant, bacteria, influent.

INTRODUCTION

Rapid industrialization has necessitated the manufacture and use of different chemicals in day to day life (Shah, 2014). Approximately, 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually, worldwide (Shah, 2014). Pollution due to textile industry effluent has increased during recent years. Moreover, it is very difficult to treat textile industry effluents because of their high biochemical oxygen demand, chemical oxygen demand, heat, color, pH and the presence of metal ions (Shah, 2014). Activated sludge constitutes a crucial tool in the biodegradation of organic materials, transformation of toxic compounds into harmless products and nutrient removal in wastewater treatment plants (WWTPs). It contains a

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highly complex mixture of microbial populations whose composition has been intensively studied in the past decades. By applying culture-dependent methods many species have been isolated from activated sludge (Dias and Bhat, 1964; Prakasam and Dondero, 1967; Benedict and Carlson, 1971).

However, a great majority cannot be obtained by conventional techniques (Wagner et al., 2002) and, consequently, current molecular techniques such as sequence analysis of 16S rRNA gene clone libraries (Snaidr et al., 1997), fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE) (Boon et al., 2002), thermal gradient gel electrophoresis (TGGE) (Eichner et al., 1999) and terminal restriction fragment length polymorphism (Saikaly et al., 2005) along with fluorescence *in situ* hybridization (FISH) have been employed in wastewater microbiology to analyse and compare the microbial structure of activated sludge. Recently, PCR-based 454 pyrosequencing has been applied to investigate the microbial populations of activated sludge in different WWTPs as well as in full-scale bioreactors (Sanapareddy et al., 2009; Kwon et al., 2010; Kim et al., 2011; Ye et al., 2011; Zhang et al., 2011a; b), greatly expanding our knowledge on activated sludge biodiversity. An important process in WWTPs is nitrification, in which ammonium is removed by converting it first into nitrite and then to nitrate. Different bacterial species involved in this process have been characterized by means of clone library analysis in addition to FISH (Juretschko et al., 1998; Purkhold et al., 2000; Daims et al., 2001; Zhang et al., 2011b). Several ammonia-oxidizing and nitrite oxidizing bacterial populations belonging to the phylum *Nitrospira* and to *Beta*- and *Gammaproteobacteria* have been identified as key members in this process, such as the genera *Nitrosomonas*, *Nitrobacter*, *Nitrospira* and *Nitrosococcus* (Wagner et al., 2002; Zhang et al., 2011b).

Nevertheless, most studies of microbial diversity in WWTPs refer to freshwater plants, either domestic or industrial, and yet very little is known about plants that utilize seawater for their operation, mainly because there are still very few of these running in the world. Their utilization responds to the deficiency in hydric resources prevailing in their locations and their use will probably increase in the near future due to water shortage associated to global warming as many areas are experiencing today (Barnett et al., 2005). As a consequence, knowledge of the microbial diversity becomes crucial to identify the key players in these systems.

Many studies have focused on the analyzing the relations between the bacterial communities, especially nitrifiers, in biomass, and a type of wastewater (Limpiyakorn et al., 2011; Whang et al., 2009) in different bioreactors (Wan et al., 2011; Ye et al., 2011). There is scarcity of literature on the influence of the full-scale wastewater treatment plant organization on the bacterial consortia in biomass. Therefore, the goal of the study was a robust statistical analysis of the dependence between the presence of the particular tanks and processes in the biological treatment line of WWTPs, the influent characteristics and the community structure of total, nitrifying and denitrifying bacteria in the activated sludge. The relations between the microbial assemblages and the technological data were investigated using the canonical correspondence analysis and correlation matrix.

The main purpose of carry out this study is mainly because several chemical industry's raw waste water is coming to the common effluent treatment plant and as a result of treatment, what exactly these effluent affects is the microbial diversity of activated sludge present in the biological system of common effluent treatment plant.

MATERIALS AND METHODS

Sampling and DNA extraction

Biomass samples were collected from Effluent Treatment Plant from Aeration Tank, Secondary Clarifier and Recycle Sump. The bioreactor consists of anaerobic, anoxic, and aerobic basins for enhanced removals of nitrogen and phosphorus as well as organic matter. In the aerobic basin, a fixed synthetic mesh made from acryl and polyether was incorporated. Grab samples were collected from the aerobic basin. A bucket-type sampler was used for the suspended microorganisms, whereas a sterilized scissor was used to cut off a part of the fixed synthetic mesh, after lifting the mesh, for the attached microorganisms. Samples were immediately frozen before being transported to the laboratory. Genomic DNA was extracted from 1.5 ml of suspended microorganisms and 1.5 g of fixed synthetic mesh, in duplicate, using the Ultra Clean soil extraction kit following the manufacturer's protocol. Five of the WWTPs, 1, 2, 3, 4, 5 received industry and domestic wastewater. The remaining WWTPs, 6, 7, 8, 9 treated domestic wastewater.

PCR amplifications

Bacterial 16S rRNA gene fragments were PCR-amplified using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 518R (5'-ATTACCGCGGCTGCTGG-3'). Each 50 µl PCR reaction included 1× EF-Taq buffer (Bangalore Genei, India), 2.5 units of EFTaq polymerase, 0.2 mM dNTP mix, 0.1 µM of each primer, and 100 ng of template DNA. The PCR temperatures were as follows: 95°C for 10 min; 35 cycles consisting of 94°C for 45 s, 55°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 10 min. The duplicate PCR products were pooled and purified using the QIA quick gel extraction kit (Bangalore Genei, India). The purified PCR products were used for the following cloning and Sanger Sequencing.

Cloning and Sanger sequencing

The purified PCR products were cloned using the pGEM-T Easy Vector System (Promega, Madison, USA) according to the manufacturer's instructions. Randomly picked transformed *Escherichia coli* clones were transferred to 96-well plates and sequenced using the T-7 primer on ABI 3730XL sequencers (Applied Biosystems, USA) by Macrogen.

Table 1. Primers used and denaturing gradient gel electrophoresis condition.

Amplicon	Specificity	Primer set	PCR product length (bp)	Reference	Denaturant percentage; gradient of gel (%)	References
16S rDNA	V3 region within the bacterial 16S rDNA	341F/515R	~230	Muyzer et al. (1993)	30-60 6	For This research
nosZ gene	Nitrous oxide reducing bacteria	NosZ1/NosZ2	~500	Kloos et al. (2001) Throba¨ck et al. (2004)	40–70 7	For This research
amoA gene	Ammonia oxidizing bacteria	301F/302R amoA-1F/ amoA-2R	~700 ~500	Norton et al. (2002) Rotthauwe et al. (1997)	30–60 6	Cydzik-Kwiatkowska et al. (2011)

Table 2. Numerical values assigned to express the number of separate biological processes and the presence of the tanks favouring denitrification in WWTP treatment line.

Parameter	Process/ denitrification tanks	Numerical value
Process	Nitrification; carbon removal	1
	Nitrification; denitrification; carbon removal	2
	Phosphorus removal; nitrification; carbon removal	2
	Phosphorus removal; simultaneous nitrification/denitrification; carbon removal	2.5
	Phosphorus removal; nitrification;denitrification; carbon removal	
Denitrification tanks	Lack of separate denitrification tank	0
	Pre-denitrification tank	0.5
	Post-denitrification tank	0.5
	Simultaneous nitrification/denitrifiction tank	0.75
	Seperated denitrification tank	1

Polymerase chain reaction–denaturing gradient gel electrophoresis

The biomass from Effluent Treatment Plant was sampled twice from aerobic tanks and was frozen in -20°C prior to molecular analysis. DNA was extracted from approximately 400 mg of centrifuged sample using a Fast DNA® SPIN Kit®. The working solutions with the DNA concentration of 50 ng/lL were prepared and the concentration of the DNA was measured spectrophotometrically using Bio Photometer (Eppendorf, Germany). The PCRs were performed in an Eppendorf® Mastercycler Gradient (Eppendorf). The determination of the total bacteria diversity was based on 16S rDNA analysis with a primer set 341F/515R. The first-phase nitrifiers' diversity was based on the analyses of amoA gene (301F/302R and amoA-1F/amoA-2R primer sets) that codes for the ammonium monooxygenase involved in the ammonium oxidation to nitrite. The denitrifying bacteria diversity was assessed based on the presence of nosZ gene (NosZ1/NosZ2 primer set), which codes for the nitrous oxide reductase responsible for the last step of denitrification (the gene is present in bacteria conducting full denitrification). The PCR mixture contained 1.7 ng/lL of extracted DNA, 0.5 lM of each primer, 100 lM of deoxynucleoside triphosphate mixture, 1.5 U of GoTaq® DNA Polymerase, 6 lL of 10 9 reaction buffer supplied with polymerase, 1.5 mM $MgCl_2$ and sterile water to a final volume of 30 lL. The amplification of the amoA gene was performed as a nested-PCR (Cydzik-Kwiatkowska and Wojnowska-Baryła, 2011). The thermal profile for the 16S rDNA amplification was: 94°C for 5 min, 35 cycles of: denaturation at 94°C for 45 s, annealing at 62°C for 45 s, extension at 72°C for 1 min, and a final elongation at 72°C for 5 min. The thermal profile for the nosZ gene amplification was: 94°C for 5 min, 6 cycles of touchdown PCR (denaturation 94°C for 30 s, annealing for 1 min with an 1°C for two cycles decrement at temperature 61°C, extension at 72°C for 1 min), followed by 25 cycles of 94°C for 30 s, 58°C for 1 min, 72°C for 1 min and a final elongation for 10 min at 72°C. The presence of the PCR products was confirmed by agarose electrophoresis. The amplified products were resolved on DGGE gels using a dCode System (Bio- Rad, USA). The electrophoresis conditions are presented in Table 1. Denaturing gradient gel electrophoresis gels were stained with SYBR Gold and digitalized using Kodak 1D 3.6 Image Analysis Software. The amoA amplicons that were clear and had a high intensity were excised from the DGGE gel, reamplified and sequenced at Bangalore Genei, India.

Statistics

In all tests, the significant effects were those with p value <0.05. For the calculations, the numerical values were assigned to express the number of separate biological processes realized in the biological treatment line of WWTPs and the presence of the tanks favouring denitrification (Table 2). The canonical correspondence analysis (CCA) was performed on the relative DGGE band intensities with the Monte Carlo method. To the CCA analysis, next to metadata obtained by DGGE, the technological data such as the COD/N and BOD/COD ratios, TKN and COD in the influent, the presence (IN+) or absence (IN-) of industrial wastewater in the influent, the

Table 3. Numerical values expressing denitrification tank presence and the number of processes realized in WWTPs taken for the CCA.

Waste water treatment plant	DT	PR
1	1	2
2	0	2
3	1.25	2.5
4	1	2
5	0.75	2.5
6	1.5	3
7	0	1
8	1	2
9	1	3

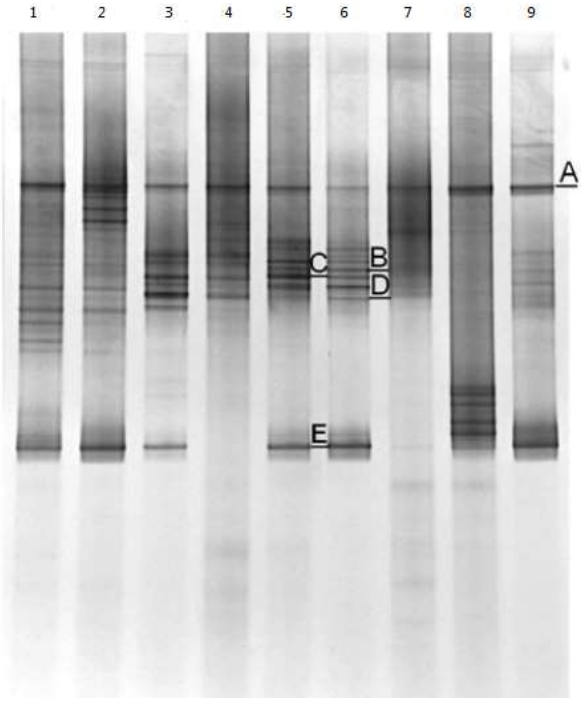

Figure 1. The DGGE analysis of PCR amplifications of the partial amoA gene. The abbreviations above each lane represent the WWTP the activated sludge samples were taken from. The bands that were sequenced are marked with capital letters.

presence of the denitrification tanks (DT) and the number of processes designed to occur in the biological treatment line of WWTP (PR) were taken. The values of DT and PR for each WWTP are presented in Table 3. The analyses were carried out using the CANOCO for Windows ver. 4.51 and CANODRAW. The correlations between the bacterial diversity and the technological parameters were analyzed using the correlation matrix in the Statistica 10.0. The statistical analysis assumed that the values from 0.9 to 1.0 point out to almost full correlation, very high correlation reaches the values from 0.7 to 0.9, strong—from 0.5 to 0.7, the medium correlation from 0.3 to 0.5, while weak from 0.1 to 0.3 (Stanisz, 2000).

RESULTS

In the present research, to analyze the bacterial assemblages, the DNA isolated from the biomass was amplified using the specific primer sets and the obtained products were separated in DGGE. In general, a higher number of bands indicate greater diversity of the analyzed microbial consortia. The DGGE separation of the amoA PCR products is presented in Figure 1, the DGGE separations of the 16S rDNA and nosZ gene are

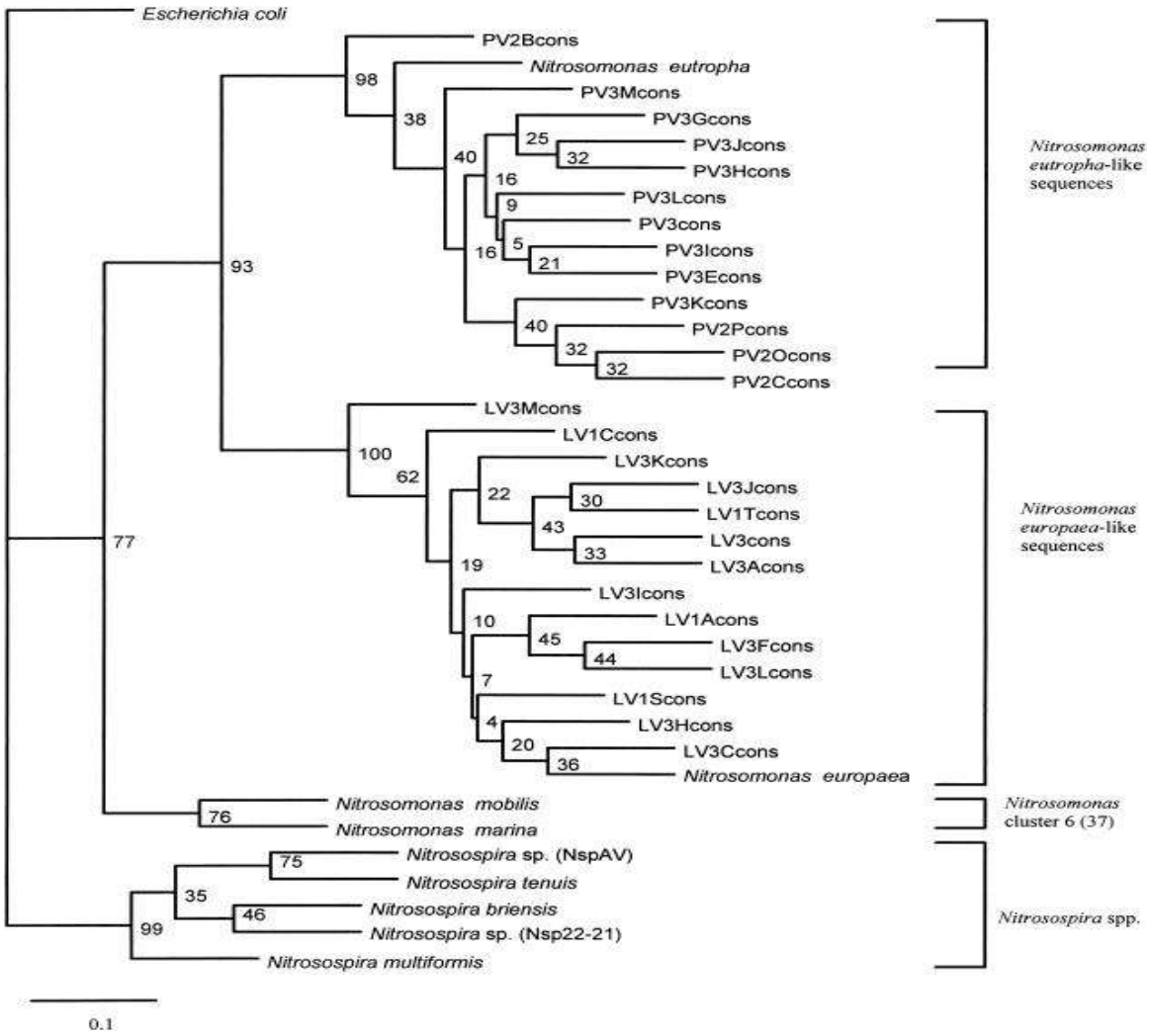

Figure 2. Phylogenetic tree showing the position within members of the class b-Proteobacteria of partial 16S rDNA sequences recovered from Buttermere lake sediments by using primers designed to amplify sequences from the b-subgroup ammonia-oxidizing bacteria.

presented in Figure 4. The sequencing of the amoA bands and the phylogenetic analysis (Figure 2) showed that most of the sequenced bands (A, B, C, D) were related to the *Nitrosospira* sp. The band E was closely related to *Nitrosomonas eutropha*. The canonical correspondence analysis is designed for relating the species composition of communities to their environment and can provide an insight into the impact of waste water treatment plant design and the operational parameters on the bacterial assemblages. The data analysis can be used in an explanatory way and it leads to an ordination diagram of samples, species and environmental variables, which optimally displays how the community composition varies with the environment. When used in a confirmatory way, it leads to statistical tests of the effects of particular environmental variables on the community composition taking into account the effect of other variables (ter Braak and Smilauer, 2002). In Figure 3a, the points represented the total bacterial consortia in different WWTPs while the environmental variables were represented by arrows. The bacterial community structure differed between the WWTPs and no separate clusters were observed in the diagram. The first axis of a biplot explained the 24% of the species-environment relation, while the summary variation explained by the two-dimensional diagram was 42%. The first and the second eigen values equaled 0.31 and 0.23, respectively. The species-environment correlations of the first two axes were very high (above 99%) showing that the measured environmental variables were sufficient to explain the major variations among the analyzed WWTPs. The correlation coefficients showed that the first axis was a presence or an absence of industrial waste water in the WWTP influent (R^2 = ± 0.71). The discrete variable IN had the highest power to explain the patterns in the species data and the significance of the explanatory

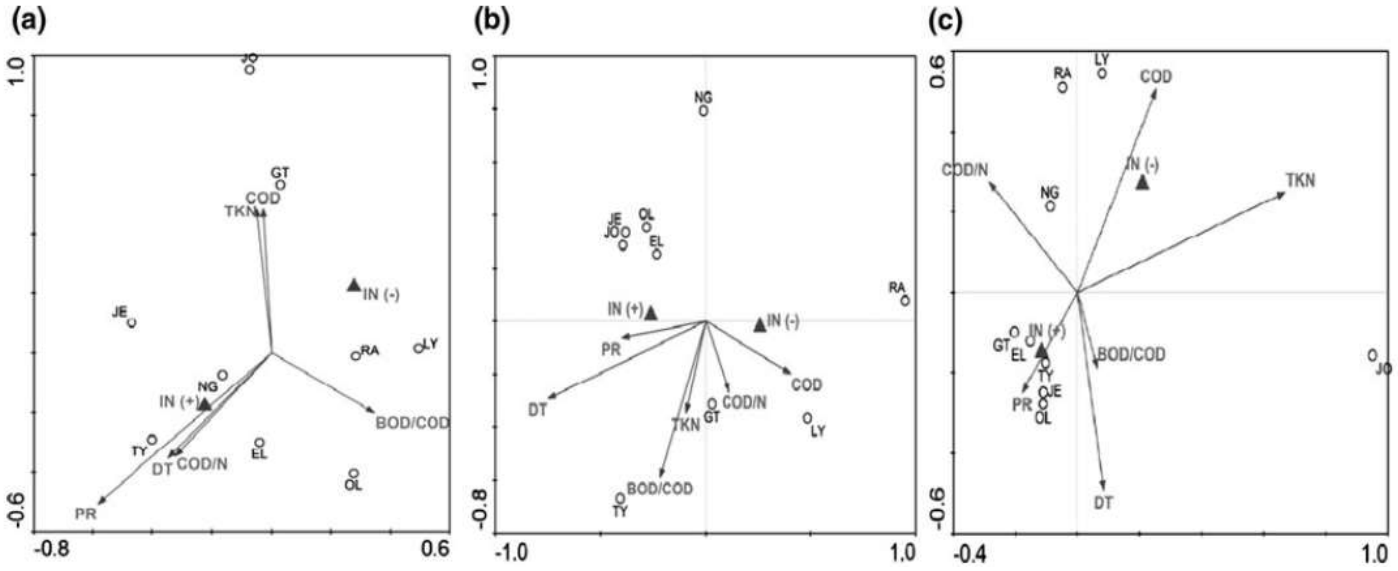

Figure 3. The CCA of a, total b, N_2O-reducing and c ammonia-oxidizing bacteria communities; the discrete (triangle) (IN ± = the presence/ absence of industrial waste water in the influent) and the continuous variables (right arrow) (the COD/N and BOD/COD ratios of the influent, COD and TKN in the influent; DT, presence of the denitrification tanks; PR, the number of the processes realized in the biological treatment line of WWTP).

effect was statistically important.

The correlations of the second axis showed a contrast between the WWTPs with the different number of processes realized in the treatment line (R^2 = 0.50) and different TKN and COD concentrations in the influent (R^2 = 0.48). The length of an arrow representing an environmental variable is a measure of how much the total bacteria community structure differs along that environmental variable. Since the PR arrow was the longest one, the number of processes realized in the WWTPs was the most important continuous environmental variable influencing the total bacteria community structure in activated sludge from all analyzed. From Figure 3b it can be concluded that the points representing the N_2O-reducing bacteria communities in 2, 7, 5 and 3 were similar and grouped together in the diagram. The first and the second axes explained 36 and 20% of the species-environment relation with the respective eigen values of 0.41 and 0.24. The species-environment correlations for the first axis were 0.98 while for the second one it equalled 0.99. The correlation coefficients showed that the first axis is DT in the WWTP treatment line (R^2 =-0.73).

This environmental continuous variable had the highest power to explain the DGGE patterns of denitrifiers. The correlations of the second axis showed that the BOD/COD ratio of the influent was the second environmental variable influencing mostly the N_2O-reducing bacteria communities in activated sludge from the analyzed WWTPs (R^2 = -0.58). The CCA analysis of the DGGE patterns characterizing the AOB communities showed that the most similar assemblages were in 6, 3, 1, 2 and 5 (Figure 3c). The first axis of a biplot explained the 30% of the species-environment relation, while the summary variation explained by the two-dimensional diagram was high and equalled 53%. The first and the second eigen values equalled 0.57 and 0.45, respectively. The species-environment correlations of the first two axes were above 99%. The correlation coefficients showed that the first axis is concentration of TKN in the influent (R^2 = 0.67) and that this factor was the most important continuous environmental variable. The second axis was the discrete variable namely the presence/absence of industrial waste water in the influent (IN; R^2 = ± 0.65). Figure 4 presents the microbial diversity expressed as the average number of amplicons in the DGGE patterns. The number of 16S rDNA bands varied depending on the WWTP. The highest number of amplicons (47) was in 1, the WWTP with a technological line ensuring the removal of carbon, nitrogen and phosphorus with the respective efficiency of 96, 86 and 95%. The lowest number of 16S rDNA amplicons was obtained in 6. Three facilities (2, 8, 6) operated without the separated denitrification tanks had the lowest diversity of denitrifiers (13, 16 and 15 nosZ bands, respectively) in activated sludge.

The highest diversity of denitrifiers (from 26 to 32 nosZ bands) was obtained in activated sludge from 3, 1 and 4. The highest diversity of AOB (ca. 27 bands) was noted for the biomass from 7 and 9. The lowest ammonia-oxidizers diversity characterized activated sludge from 6. The correlation matrix was constructed to find the dependences between the diversity of particular groups of bacteria (total, N_2O-reducing and ammonia-oxidizing)

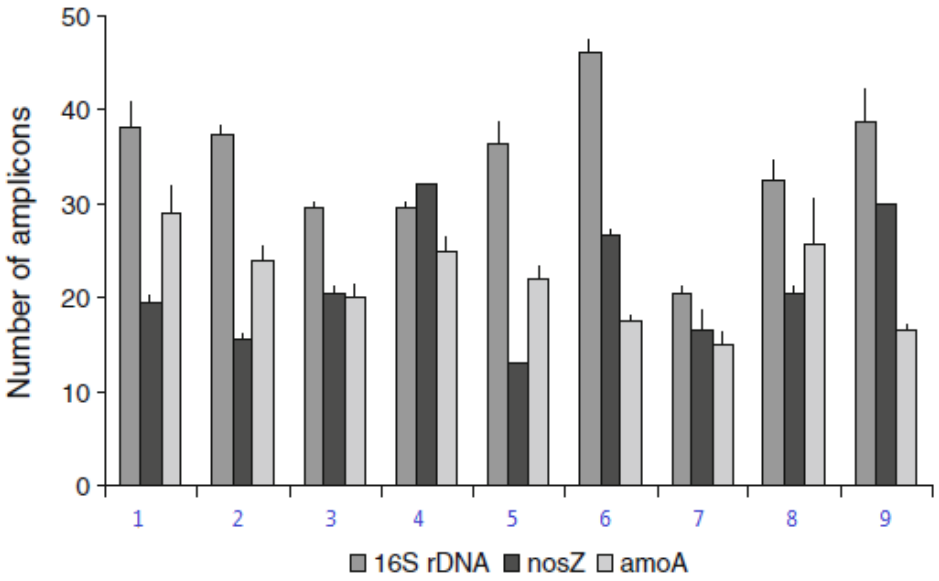

Figure 4. The average number of 16S rDNA, nosZ, amoA bands in the DGGE patterns characterizing activated sludge communities from analyzed WWTPs; the averages of two different measurements, standard deviations are given.

and the investigated WWTP design and the influent composition. It was observed that the total bacteria diversity was strongly correlated with the COD/N ratio (R^2= 0.84) and the number of processes realized in the facility (R^2 = 0.79). We also obtained a positive strong correlation (R^2 = 0.50) between the 16S rDNA band number and the presence of denitrification tank. As to the facility design, the presence of anoxic tanks had the greatest impact on the diversity of N_2O-utilizing denitrifiers (R^2 = 0.57). Analyzing the wastewater characteristics, both the presence of the industrial stream and the high COD/N ratio in the WWTP influent influenced the species richness of denitrifiers (R = 0.51 and R = 0.48, respectively). The AOB diversity was weakly positively correlated with the TKN concentration in the influent (R^2 = 0.25) and negatively influenced by the presence of industrial waste water in the influent (R^2= -0.25).

DISCUSSION

Activated sludge from nine WWTPs was investigated to determine the influence of the complexity of the biological treatment line and the influent characteristics on the structure and diversity of microbial consortia. For the statistical analysis, the CCA and correlation matrix were applied. The species composition of the total bacterial communities in activated sludge was influenced by both the design of the treatment line and the waste water characteristics. The CCA analysis showed that the number of processes realized in the WWTPs was the most important of all continuous environmental variables deciding about the structure of total bacteria assemblages. The complexity of the treatment line positively affected the overall diversity of bacteria in the biomass. The highest number of different bacterial species was noted for TY with a complex, many-stage technological line. Reversely, the lowest number of 16S rDNA bands characterized activated sludge community in 6, the smallest of all analyzed WWTPs (PE = 2,405) with the simplest biological treatment line consisting only of the aeration tank. It can be concluded that the presence of many tanks with the different oxic conditions in the treatment line favors the growth of multispecies microbial consortia thus promoting the stability of the purification processes. As to the wastewater composition, the statistically significant influence of the industrial waste water presence on both the structure and the diversity of the total bacteria communities were proven. Since the origin of industrial waste water in the analyzed treatment facilities was broad, it was difficult to claim which components of the sewage promoted the biodiversity. Our results, however, pointed out that the presence of the industrial stream favoured species richness of total bacteria in activated sludge due to the accessibility of a broader range of substrates as compared to that present in typical domestic waste water.

The WWTPs are one of the major sources of organics. Many parameters such as a substrate concentration, a C/N ratio, a type of carbon source, a nitrite accumulation and an NO concentration influence the N_2O production (Adouani et al., 2010). The knowledge about the links between the waste water treatment line design and the structure of N_2O-utilizing bacteria communities in the

biomass would allow engineers to apply the purification strategy favouring the effective reduction of the greenhouse gas emission. The research proved that the number of separated denitrification tanks in the WWTPs was the major factor deciding about the species composition and the diversity of the N_2O reducing microorganisms in activated sludge. In three facilities (2, 8, 6) operated without the separated denitrification tanks, the lowest species richness of denitrifiers in the biomass was noted. In 2, the tank with the simultaneous nitrification/denitrification was exploited meaning that nitrifiers and denitrifiers co-existed in activated sludge; however, the alternating oxic conditions did not favor the full denitrification toN2 since the activity of bacteria that possess the nosZ genes is inhibited in the presence of even low oxygen concentrations (Hochstein et al., 1984). For comparison, in 5, nitrogen removal was realized by SND in a carrousel-type bioreactor; however there was an additional tank for pre-denitrification favouring the growth of more diverse assemblage of the N_2O-utilizing denitrifiers. The highest diversity of denitrifiers was obtained in activated sludge from 3, 1 and 4. These WWTPs were characterized by both the presence of separate anoxic tanks and the highest ratio of COD/N(12–14) that was also proven to positively influence the species richness of investigated bacteria (R = 0.48).

The microorganisms capable of denitrification belong to a broad variety of groups and encompass a wide range of the physiological traits. Most denitrifiers are the aerobic heterotrophic organisms that transfer redox equivalents from the oxidation of a carbon source to N oxides under the anaerobic conditions. The ability to reduce N_2O by the nitrifying and phosphorus accumulating bacteria is also documented (Zumft, 1997). This can explain the tendency observed in the current research that more processes realized separately in the treatment line favoured the diversity of N_2O-reducers. Siripong and Rittman (2007) investigated the AOB communities in activated sludge in seven typical single stage municipal plants. Among the analyzed factors (flow rate, influent and effluent BOD and TKN, effluent ammonia, nitrite and nitrate, pH, and sewage temperature), only the seasonal temperature variations seemed to change the nitrifying community, especially the balance between *Nitrosospira* sp. and *Nitrosomonas* sp., although both genera coexisted in winter and summer samples. Lydmark et al. (2007) showed that ammonium concentration was an important structuring factor for an AOB community. In the present research, the CCA proved that the main variable influencing the AOB consortia in activated sludge was the presence of the industrial stream in the WWTPs influent. In fact, the IN variable was the only one that had a statistically significant impact on the species composition of the AOB. These results are well depicted in Figure 3c. The DGGE patterns characterizing activated sludge from6, 3, 1, JE and 5 facilities with the lower values of COD and TKN in the influent (except from 1) and the presence of industrial influent (except from 6) were grouped together in a separate cluster. The coexistence of various nitrifiers in WWTP is an evidence of a functional redundancy, a feature that may help in maintaining the stability of the system for nitrification. Wang et al. (2010) investigated the communities of microorganisms in activated sludge of eight waste water treatment systems and suggested the negative impact of the presence of industrial waste water in the influent on the nitrifiers' diversity. Our observations pointed out to a weak negative correlation between the industrial waste water presence and the species richness of AOB (R = -0.25), nevertheless, the highest diversity of these bacteria was noted in biomass from 7 and 9 that received only domestic wastewater. The lowest AOB diversity characterized activated sludge from 6, the WWTP with the simplest technological line consisting of only an aeration tank. It can be concluded that in this system, under a stable oxygen concentration, the microorganisms underwent a strong selective pressure favouring the growth of only a few best adapted species.

Whang et al. (2009) evaluated the nitrifying community and the nitrification performance of the full-scale municipal (20 mg N/L) and swine (220 mg N/L) WWTPs. Authors observed dissimilar nitrifying populations prevailing in these two plants and related this fact to different input nitrogen concentrations. In our research, in the analyzed range of the influent TKN (62 ± 14–129 ± 18 mg/L), only a weak correlation between this parameter and the AOB diversity was proven. In general, the members of the *Nitrosospira* spp. or/and the Nitrosomonas oligotropha clusters are the dominant AOB in the ammonia-low environments, whereas the members of the *N. europaea*–Nitrosococcus mobilis cluster comprise the majority of AOB in the ammonia-rich environments (Limpiyakorn et al., 2005). The sequencing of the chosen amoA bands and the phylogenetic analysis showed that the AOB in the analyzed activated sludge samples were related to both *Nitrosomonas* sp. and *Nitrosospira* sp. (Figure 2). An interesting observation can be made on band E, obtained from *N. eutropha*. This species was present in all the WWTPs except from 3, in 6 only a weak band was observed. N. eutropha is commonly found in strongly eutrophic environments such as the municipal and industrial sewage disposal systems. This microorganism has a high tolerance for both elevated ammonia concentrations and the fluctuating conditions (especially oxic/anoxic cycles) (Koops et al., 1991; Stein et al., 2007). The low number of *N. eutropha* in 6 (DGGE is a semi-quantitative technique so such a statement is justified) can be explained by the simplicity of the biological treatment line in this WWTP—it consisted of only an aeration tank. The stable oxic conditions and a lack of environmental fluctuations did not favour the growth of the analyzed species. The 3 WWTP, on the other hand, received waste water from a wood industry that did not use water as an input for the manufacturing processes but generated several waste water streams

after washing/cleaning procedures. In the production of wood-based floors and wood-laminates, small volumes of highly polluted waste waters with the high contents of formaldehyde and COD are generated (for example cleaning of machines that are used to apply urea–formaldehyde resins onto wood-fiber boards) (Kaczala et al., 2010). Waste water with formaldehyde could have negatively influenced the AOB community in 3, especially *N. eutropha*, since its genome lacks genes for urease metabolism (Stein et al., 2007).

Conflict of interests

The author did not declare any conflict of interest.

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