COMPARATIVE ASSESSMENT OF DENITRIFICATION / NITRIFICATION PROCESSES IN WATER TREATMENT TECHNOLOGIES IN WWTP "SADINATA" AND WWTP "KUBRATOVO"

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Abstract: The technology on base of denitrification / nitrification (D/N) is widely applicable in biological treatment of municipal and industrial wastewaters as well as of leachate from municipal solid waste landfills. The aim of this study is to make a comparative assessment between denitrification / nitrification processes in two types of wastewater treatment plants and on this basis to differentiate the key factors for their management. The samples were taken from the biobasins in denitrification and nitrification zone from WWTP "Kubratovo" (for treatment of municipal and some industrial wastewaters of Sofia City) and WWTP "Sadinata" (for treatment of leachate from municipal solid waste landfill of Sofia City). Key physicochemical (pH, dissolved oxygen, organics concentration, concentration of inorganic nitrogen and phosphorus), technological (C:N:P, BOD_5:COD, sludge volume index, filamentous index, relative filament abundance), microbiological (quantity of aerobic heterotrophs, denitrifying and nitrifying microorganism) and enzymological (total dehydrogenase activity and nitrate reductase activity) indicators have been investigated and comparatively analyzed.

The obtained results showed that denitrification was the critical process in the technology on base of D/N and especially for WWTP "Sadinata". The concentration of biodegradable organic matter was identified as a major factor for successful implementation of denitrification. The nitrification processes in both WWTPs were accomplished with high intensity.

Keywords: activated sludge, denitrification/ nitrification, microbiological and enzymological control, wastewater treatment

INTRODUCTION

The technology on base of denitrification / nitrification (D/N) is widely applicable in biological wastewater treatment. It is applied for treatment of water with high concentration nitrogen-containing organics (wastewater from food industry with high protein concentration) or with high concentration inorganic nitrogen (landfill leachate with high ammonium concentration). This technology is also appropriate for wastewater requiring specialized module for detoxification of xenobiotics (wastewater from textile industry with high concentration of azodyes) which biodegradation is associated with release of nitrogen compounds. Conventional technology on base of D/N is applied in biological treatment of municipal and industrial wastewaters as well as of leachate from municipal solid waste landfills. An important attention should also be paid to the fact that it is also applied as a part of the technologies for simultaneous elimination of carbon, nitrogen and phosphorus from wastewater (Nitrification Denitrification Biological Excess Phosphorus Removal or NDBEPR). Despite the existence of numerous scientific articles on the optimization of denitrification and nitrification processes and the numerous model experiments [4, 6, 8, 12, 13, 14, 16], often there are problems in the managing these biological processes in the wastewater treatment plants [5, 8, 9].

Nitrification is biological aerobic process of oxidation of ammonium to nitrite (first phase of nitrification) and oxidation of nitrite to nitrate (second phase of nitrification). The first phase of the process is performed by ammonia - oxidizing bacteria including *Nitrosomonas sp.*, *Nitrosospira sp.*, etc. The second phase of nitrification is performed by nitrite - oxidizing bacteria such as *Nitrobacter sp.* and *Nitrococcus sp.* According some authors there are several operational conditions that affect the activity and growth of nitrifying bacteria in wastewater treatment plants, which are: mean cell residence time; temperature; dissolved oxygen; pH; alkalinity; toxicity, high biochemical oxygen demand (BOD_5) in influent and phosphorus deficiency [4, 8, 9]. The accumulation of nitrite is an example for incomplete nitrification caused from limiting factors as low dissolved oxygen, pH changes, high BOD_5, and inhibition [9].

Denitrification is biological process of nitrate reduction (reduction of nitrate to nitrogen or dinitrogen oxide) which is occurs under anoxic conditions by facultative anaerobic bacteria. Denitrifying bacteria degrade soluble BOD_5 during nitrate reduction. Commonly reported genera of denitrifying bacteria include *Alcaligenes sp.,* *Achromobacter sp., Bacillus sp*. and *Pseudomonas sp*. [4, 8]. The main operational conditions that affect the denitrification in wastewater treatment plants are: abundance and activity of denitrifying bacteria; temperature; nutrients; oxidation-reduction potential; denitrification time; soluble biodegradable organics measured as BOD_5, toxicity [9].

Which are the key factors for each process and for quantity and activity of denitrifying and nitrifying bacteria are the important questions for successful management during exploitation of the wastewater treatment plants (WWTPs). The aim of the current study is to make a comparative assessment between denitrification / nitrification processes in WWTP "Kubratovo" and WWTP "Sadinata", and on this basis to differentiate the key factors for their management.

MATERIALS AND METHODS

The samples were taken from the biobasins in denitrification and nitrification zone from WWTP "Kubratovo" (for treatment of municipal and some industrial wastewaters of Sofia City) and WWTP "Sadinata" (for treatment of leachate from municipal solid waste landfill of Sofia City). Key physicochemical (pH, dissolved oxygen, organics concentration, concentration of inorganic nitrogen and phosphorus), technological (C:N:P, BOD_5:COD, sludge volume index, filamentous index, relative filament abundance), microbiological (quantity of aerobic heterotrophs, denitrifying and nitrifying microorganisms) and enzymological (total dehydrogenase activity and nitrate reductase activity) indicators have been investigated and comparatively analyzed.

The plug flow biobasins in WWTP "Kubratovo" is separated into 3 sections: anaerobic, anoxic and aerobic, in which the simultaneous removal of carbon, nitrogen and phosphorus is accomplished. The first critical control point for sampling in this plant was in the anoxic zone, where predominantly occurs denitrification. The second critical control point for sampling was in aerobic zone where predominantly occurs nitrification processes. The biological module in WWTP "Sadinata" has two stages. First of them is a preliminary stage of denitrification and nitrification. The second stage includes treatment in sequencing batch reactors (SBRs) on basis of denitrification and nitrification. Each SBR passes through: fill phase, react phase (under anoxic or aerobic conditions), settle phase, decant phase and idle phase. Samples were taken from two SBRs during react phase but the first sample point is when the reactor is in the denitrification phase and the second is in the nitrification phase. Samples from both WWTPs were taken during March, 2014.

Data for pH and dissolved oxygen was kindly provided by the WWTPs. The chemical indicators as biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), ammonium, nitrites, nitrates, phosphates were analyzed according to APHA [1]. BOD_5 and COD were determined of a mixed sample from the biobasins (including activated sludge and water). BOD_5:COD provides information about the share of biodegradable organic matter to the total quantity of organics, measured as COD.

Activated sludge structure from both WWTPs was analyzed by microscope. Standard method for the examination of the settleability of activated sludge was used for measurement of sludge volume index (SVI) [3]. Filamentous index (FI) was analyzed according Sezgin et al. [15] and relative filament abundance was investigated according to Jenkins et al. [10]. Mixed liquor suspended solids in activated sludge samples were analyzed according to standard method for calculation of SVI and FI [3].

Key groups of microorganisms (aerobic heterotrophs, denitrifying and nitrifying bacteria) were investigated by cultivation techniques on solid or in liquid mediums. Aerobic heterotrophs (AH) were cultivated on Nutrient Agar for 2 days. Denitrifying microorganisms (Dn) were counted on Giltay medium for 14 days and nitrifying bacteria (Nitr) were cultivated in liquid medium of Saratchandra for 14 days [17]. The denitrifying bacteria were cultivated under anaerobic conditions in anaerobic jars with addition of Anaerocult A (Merck & Co., Inc.). All microbial groups were incubated in thermostat at $28\pm2^{o}C$. The activity of total aerobic dehydrogenase (DHA) was used as an indicator for the total metabolic activity of activated sludge (AS). It was measured spectrophotometrically by use of 2,3,5-triphenyltetrazolium chloride [7]. Nitrate reductase activity (NRA) was used as an indicator for denitrifying activity of AS and it was studied according to method described from Topalova [17]. DHA and NRA were presented as μMol/min.mg protein and protein concentration in the cell extracts was measured according to Kochetov [11]. All data are average values from at least three independent repetitions and standard deviations are shown on figures as error bars.

RESULTS AND DISSCUSION

Microbiological and enzymological indicators of activated sludge in denitrification and nitrification zones of WWTP "Kubratovo" and WWTP "Sadinata":

One of the important factors for the implementation of the denitrification and nitrification processes is the quantity and activity of the key microbial groups. The quantity of aerobic heterotrophic microorganisms, denitrifying and nitrifying bacteria for both WWTPs is presented on Fig. 1. The comparative assessment showed that their amount was significantly higher in WWTP "Kubratovo" which suggested more appropriate conditions for its growth and activity. The average quantity of aerobic heterotrophs was 2,6.10^7 CFU.g^{-1}, of denitrifying microorganisms was 4.10^5 CFU.g^{-1} and amount of nitrifying bacteria was 9,6.10^6 CFU.g^{-1} in both zones of the biobasins in WWTP "Kubratovo". In the same time, the average quantity of aerobic heterotrophs was 1,9.10^6 CFU.g^{-1}, of denitrifying microorganisms was 5,1.10^3 CFU.g^{-1} and amount of nitrifying bacteria was 3.10^5 CFU.g^{-1} in both SBRs in WWTP "Sadinata". The quantitative differences among the microbial groups from both WWTPs were related to the differences in the wastewater composition. The lower amount of heterotrophs in WWTP "Sadinata" was due to the lower proportion of biodegradable organic matter measured as BOD_5:COD and lower BOD_5 values (Fig. 4). Higher quantities of nitrifying microorganisms in WWTP "Kubratovo" were due to the higher concentrations of ammonium ions in the biobasins of this plant. Lower concentrations of ammonium ions in the WWTP "Satinata" can be related to the presence of a preliminary stage of denitrification / nitrification in which the inorganic nitrogen was initially removed.

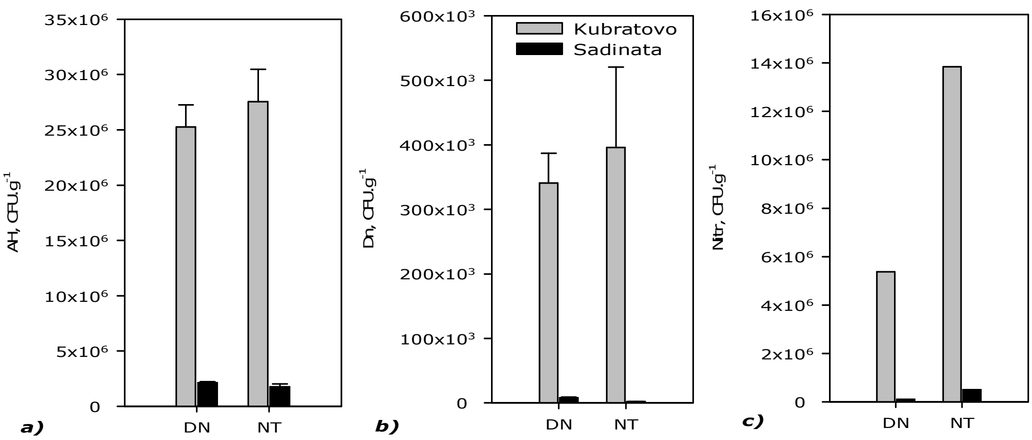

Fig. 1. Microbial groups of: a) aerobic heterotrophs (AH); b) denitrifying (Dn) and c) nitrifying (Nitr) bacteria of activated sludge in denitrification /DN/ and nitrification /NT/ zones at WWTP "Kubratovo" and WWTP "Sadinata"

In both WWTPs, the dominant group was that of the aerobic heterotrophs which was related to high organics concentration in biobasins (Fig. 4). For example, the heterotrophs (including the denitrifying microorganisms also) in WWTP "Sadinata" were in a higher amount in the denitrification zone than in the nitrification (Fig. 1a, 1b) which was related with higher concentration of biodegradable organics measures as BOD_5 (Fig. 4b). Data about nitrifying bacteria showed that in both WWTPs this group was well presented and it dominated in the nitrification zone in comparison to denitrification zone.

Data for the enzymological indicators are compared on Fig. 2. It is established that the aerobic dehydrogenase activity is 43 times higher for the activated sludge from WWTP "Kubratovo". This showed a higher biodegradation potential of AS as well as a higher concentration of biodegradable organic matter in wastewater. The lower DHA in WWTP "Sadinata" indicated that pollutants affect activity but do not completely inhibit the metabolic processes in the biological system. The obtained data for the second enzymological indicator, nitrate reductase activity (NRA), were again higher in WWTP "Kubratovo". The nitrate reductase catalyzes the denitrification processes and it can be assumed that the nitrate reduction occurred at a higher rate in WWTP "Kubratovo". In WWTP "Sadinata" nitrate reductase had a lower activity, which implied a lower rate of denitrification process.

One of the factors, affecting nitrate reductase activity in this case, was the high concentration of dissolved oxygen, especially in the denitrification zone (Fig. 3a) which inhibited the enzyme complex. Another probable factor was the low share of biodegradable organic matter (Fig. 4) and the inappropriate form of the available organics, which served as a source of protons to carry out the reduction of nitrates. Many of the denitrifying microorganisms are characterized by the absence of the necessary hydrolytic enzymes, which is why they cannot utilize some macromolecules [4, 8].

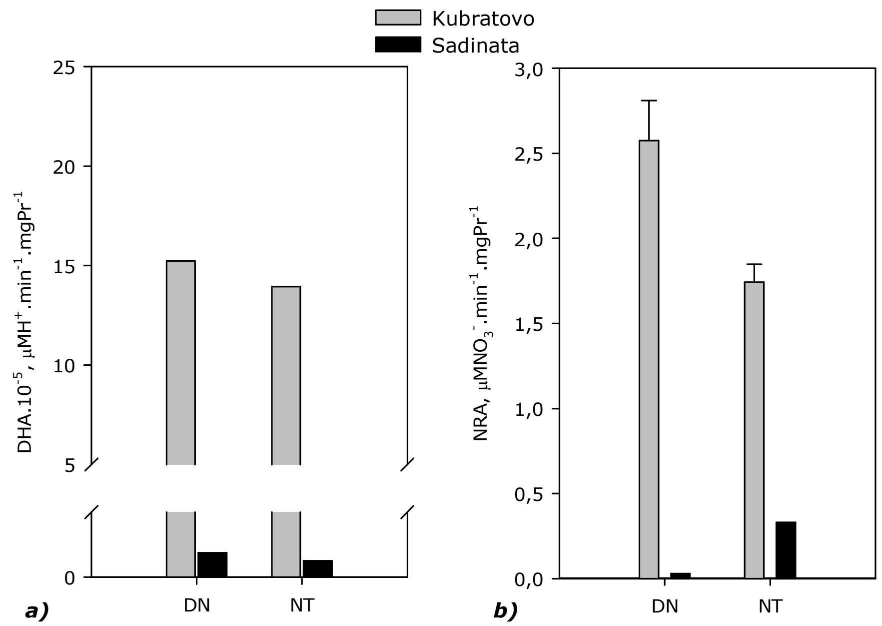

Fig. 2. Enzyme activity of: a) dehydrogenase (DHA) and b) nitrate reductase (NRA) of activated sludge in denitrification /DN/ and nitrification /NT/ zones at WWTP "Kubratovo" and WWTP "Sadinata"

The obtained results showed that quantity and activity of the key microbial groups were higher in activated sludge from WWTP "Kubratovo" which supposed that biodegradation and biotransformation processes were accomplished with higher rate and effectiveness. The lower activity of activated sludge from WWTP "Sadinata" could be related to some operational conditions during wastewater treatment or with composition of leachate. The physicochemical data were discussed in the next section to clarify the reasons for the differences in the quantity and activity of the microorganisms in both WWTPs.

Physicochemical indicators in denitrification and nitrification zones of WWTP "Kubratovo" and WWTP "Sadinata":

The concentration of dissolved oxygen and pH are presented on Fig. 3. The optimal concentration of dissolved oxygen for nitrification processes is between 2 and 3 mg/l and for denitrification processes is between 0.2 and 0.8 mg/l [9]. The oxygen concentration in WWTP "Kubratovo" was in the optimal range in both zones of biobasin. The higher concentration of dissolved oxygen (exceeded about 3 times the optimal values in denitrification zone) was an inhibiting factor for denitrification in WWTP "Sadinata". Denitrifying bacteria are facultative anaerobic bacteria and they have the enzymatic ability to use nitrates in the absence of molecular oxygen. However, they prefer the use of molecular oxygen to utilize biodegradable organics because they could generate more ATP during aerobic biodegradation processes in comparison to denitrification. Also, a higher concentration of dissolved oxygen (above 3-4 mg/l) in the nitrification zone of WWTP "Sadinata" was not necessary from economical point of view.

The optimal values of pH for most nitrifying bacteria are between 7.2 and 8.0 but some authors reported that the nitrification is successfully at a pH near to neutral (between 6.8 and 7.2) [4]. The optimal pH value for denitrification varies between 7.0 and 7.5 [9]. The potential of hydrogen (pH) was above optimal values for the processes of nitrification and denitrification (pH was above 8.1) in the denitrification zone of the both WWTPs and in the nitrification zone of WWTP "Kubratovo".

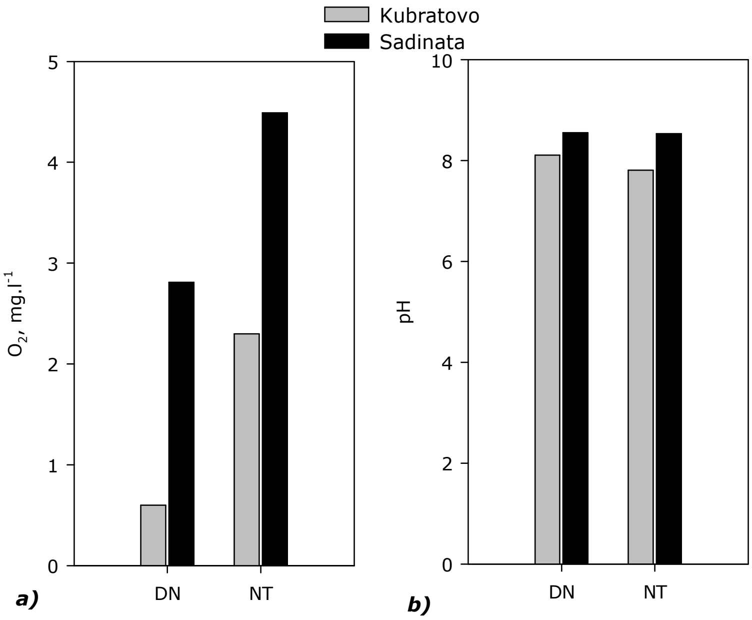

Fig. 3. Concentration of dissolved oxygen (a) and pH (b) in denitrification /DN/ and nitrification /NT/ zones at WWTP "Kubratovo" and WWTP "Sadinata"

The organics concentration was measured as COD and BOD_5, and the BOD_5:COD ratio was used as an indicator for the percentage of biodegradable organic to the total organics concentration. COD values in the both WWTPs were around 3000 $mgO_2.l^{-1}$ which included dissolved and suspended organics (Fig. 4). BOD_5 was five times higher in WWTP "Kubratovo" than in WWTP "Sadinata". The concentration of biodegradable organics in WWTP "Sadinata" was a limiting factor for denitrification process which led to decrease of the proton concentration required for the reduction of nitrates. The low BOD_5:COD showed that over 88% of COD was in the form of toxic or refractory organics which can inhibit denitrification processes during leachate treatment. Extractable C_{10}-C_{40} hydrocarbons, phenols, benzene, toluene, nicotine are some examples for toxic or refractory organic compounds in the leachate [2]. They can inhibit the activated sludge but also their biodegradation are realized at a low rate. So the released protons, result from the catabolic processes, are insufficient to carry out the nitrate reduction.

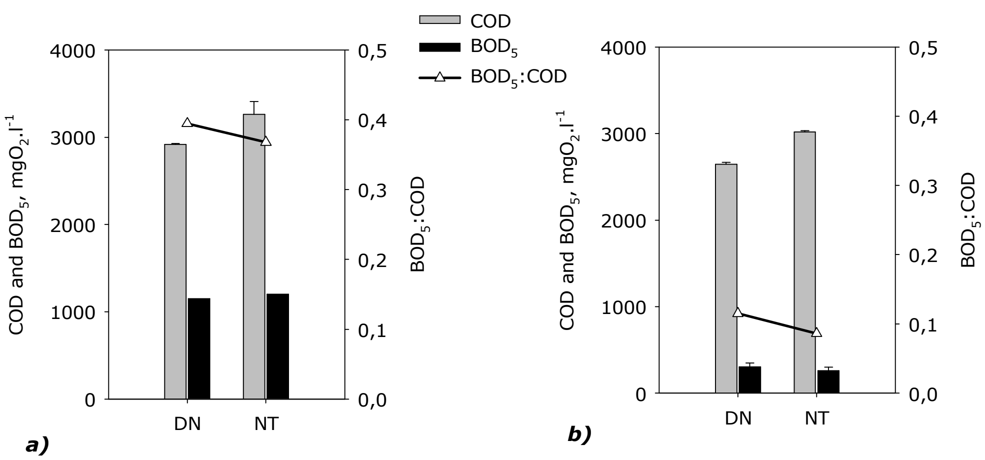

Fig. 4. Concentration of organic matter in denitrification /DN/ and nitrification /NT/ zones of: a) WWTP "Kubratovo" and b) WWTP "Sadinata"

The high concentration of nitrates in the denitrification zone of SBR in WWTP “Sadinata” indicated that denitrification was most likely inhibited (Fig. 5c). Nitrification process was occurred in the both WWTPs. It was occurred more intensively in the WWTP “Sadinata” as was determined by the higher concentration of nitrites and nitrates in the nitrification zone. The presence of a preliminary stage of denitrification and nitrification in WWTP “Sadinata” was one of the reasons for lower concentration of biodegradable organics and ammonium in biobasins.

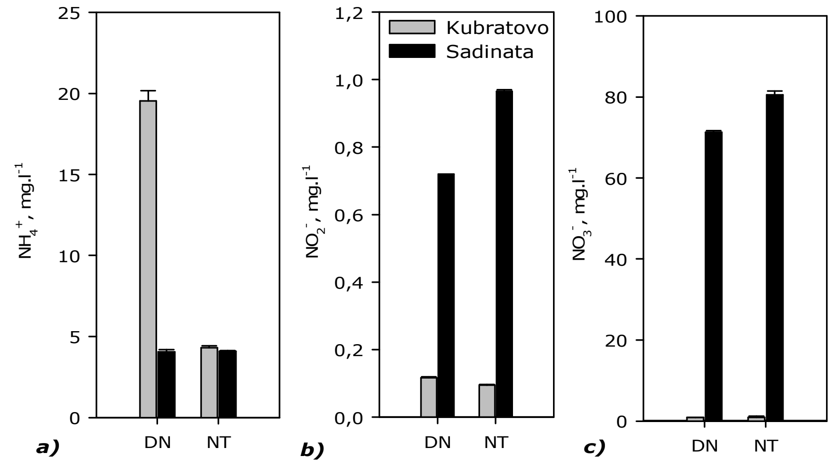

Fig. 5. Concentration of: a) ammonium ions; b) nitrites; c) nitrates in denitrification /DN/ and nitrification /NT/ zones at WWTP “Kubratovo” and WWTP “Sadinata”

The relationship between carbon, nitrogen and phosphorus was studied by key ratios between important technological indicators for denitrification processes (Table 1). They include C:N:P with optimal values 100:5:1; $BOD_5:NO_3^-$ with optimal values 3:1 and COD:N-NO_3 with optimal values 6:1 for denitrification [4, 8, 9]. In our study we calculated C:N:P as BOD_5:TCN:P-PO_4. TCN presented total calculate nitrogen and it was measured as a sum of N-NH_4, N-NO_2 and N-NO_3.

Table 1. Relationship among carbon, nitrogen and phosphorous for denitrification processes

Parameter	Optimal conditions	WWTP “Kubratovo”	WWTP “Sadinata”
C:N:P	100:5:1	399:5:1	93:6:1
$BOD_5:NO_3^-$	3:1	1300:1	4,9:1
COD:N-NO_3	6:1	397:1	42:1

It was founded that the optimal ratios between the parameters were violated in both WWTPs. In WWTP “Kubratovo” limiting factors were the low concentrations of nitrogen and phosphorus at the available concentration of biodegradable organic matter, while in WWTP “Sadinata” the limiting factor was the low concentration of biodegradable organics at the available high concentrations of nitrogen and phosphorus.

Technological indicators of activated sludge in both WWTPs: In the comparative assessment of the processes in the two WWTPs, some technological parameters of the activated sludge, which are used in the control of the wastewater treatment processes, were also analyzed. A line on the graphs showed the values whose overshoot indicates deformations in the structure of the AS. The parameter mixed liquor suspended solids was used as an indicator for quantity of biomass of activated sludge. The biomass quantity was in normal range for both WWTPs (3 g.l^{-1} for AS from WWTP "Kubratovo" and between 4-5 g.l^{-1} in WWTP "Sadinata") (Fig. 6a).

SVI indicates settleability of activated sludge and it was used as an indicator for deformations in its structure. SVI varied between 70 and 100 ml.g^{-1} at normally functioning AS [4, 9, 10]. Values above 150 ml.g^{-1} are indication for bulking of activated sludge (filamentous or zoogleal). Values below 70 ml.g^{-1} are indication for another type of deformation of the AS with pinpoint flocs prevails (or starving AS). SVI was higher in WWTP "Kubratovo" (between 246.15 and 286.74 ml.g^{-1}) than in WWTP "Sadinata" (between 179.98 and 212.26 ml.g^{-1}). Obtained data for SVI showed bulking of AS (Fig. 6b). The filamentous index was over 1.10^{7} μm.mg^{-1} in both WWTPs and it was indication for filamentous bulking of AS (Fig. 6c). The results for relative filament abundance confirmed that conclusion (Fig. 6d). The relative filament abundance varied between 4 and 5 in both WWTPs. The value 4 means that presence of filamentous microorganisms was very common in the most flocs (with a density between 6 and 20 filamentous microorganisms in a floc) [10]. The value 5 means that presence of filamentous microorganisms was high in the most flocs (above 20 filamentous microorganisms in a floc) [10].

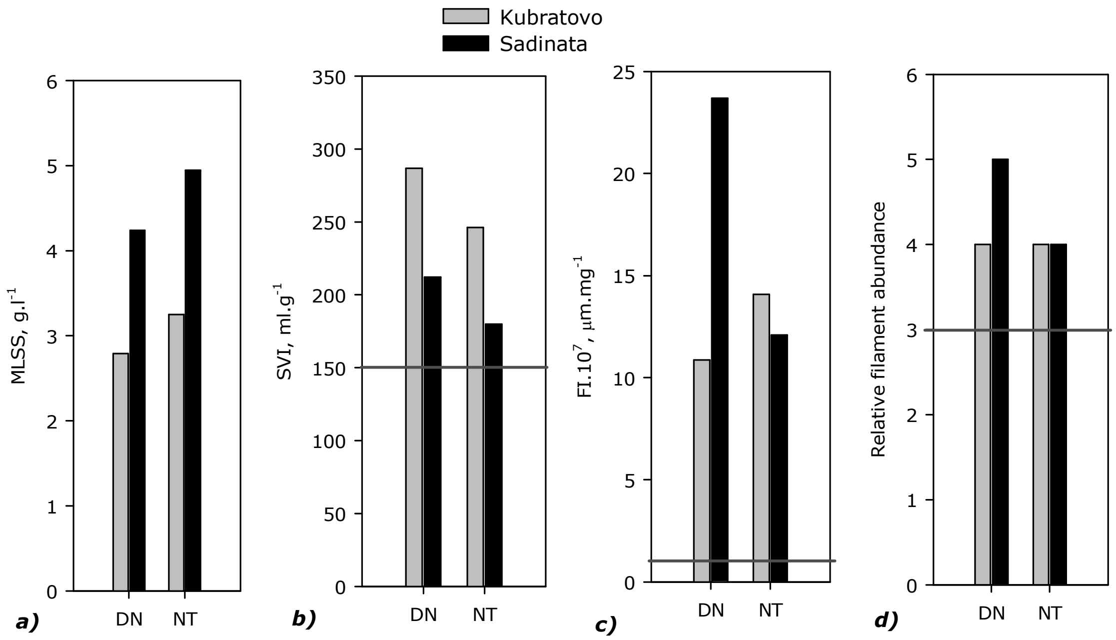

Fig. 6. Technological indicators of the activated sludge: a) mixed liquor suspended solids (MLSS); b) sludge volume index (SVI); c) filamentous index (FI); d) relative filament abundance in denitrification /DN/ and nitrification /NT/ zones at WWTP "Kubratovo" and WWTP "Sadinata"

The parameters sludge volume index (SVI), filamentous index (FI) and relative filament abundance showed availability of deformation in activated sludge structure. The bulking of activated sludge was related to domination of filamentous microorganisms. It is important to have a balance between floc forming and filamentous microorganisms because the dominance of one group over the other leads to deformations in the structure and respectively the functioning of the AS. Filamentous bacteria have a higher surface-to-volume ratio than floc-forming bacteria, and this helps them survive at low organics concentration and low nutrient concentration [4].

The microscopic analysis showed that in WWTP "Kubratovo" predominated the species *Microthrix parvicella* (Fig. 7a, 7b) and in WWTP "Sadinata" predominated the species *Sphaerotilus natans* (Fig. 7c, 7d). Some factors that determine the dominance of *M. parvicella* are: low concentrations of nitrogen, organics or the presence of slowly biodegradable substrates such as fat, and others [10]. Low water temperatures also favor the development of this species. The most likely cause of this dominant species in WWTP "Kubratovo" was the low nitrogen concentration as well as the presence of slowly degradable substrates such as fats and proteins. They are included in domestic wastewater and industrial wastewater from food processing which are discharged in municipal WWTPs.

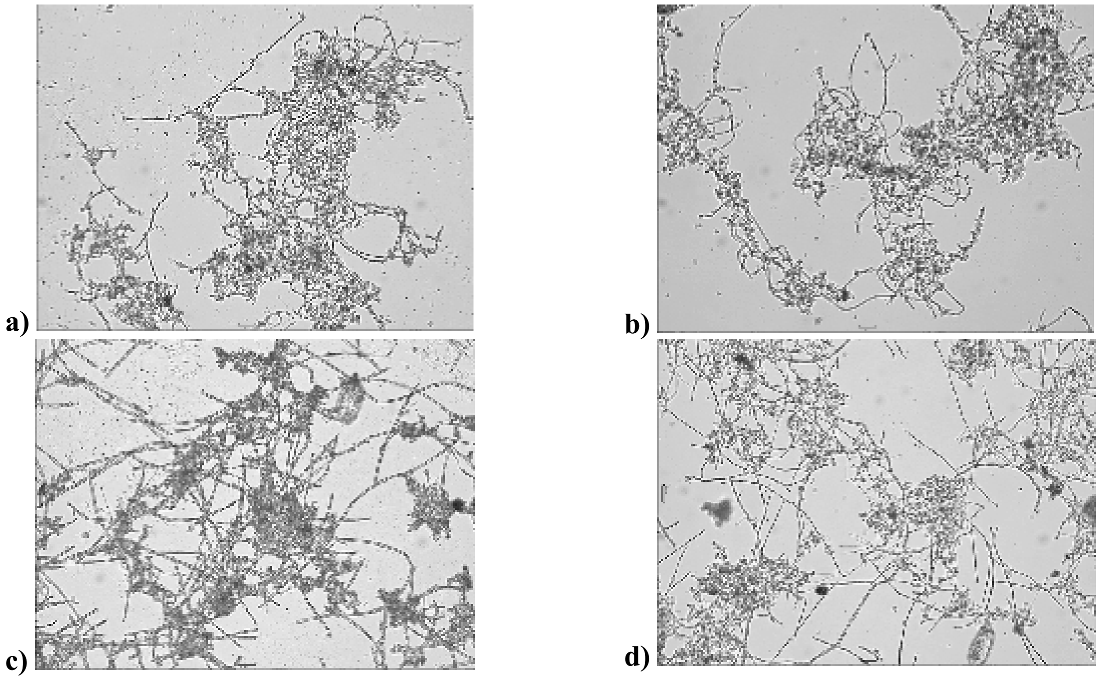

Fig. 7. Microscopic photographs (Light Microscope at magnification 400X) of activated sludge from: a) denitrification and b) nitrification zone at WWTP "Kubratovo"; c) denitrification and d) nitrification zone at WWTP "Sadinata"

S. natans is presents in low quantity in normal functioning activated sludge [4, 10] but it was presented with higher quantity in WWTP "Sadinata". Some causes for the domination of *S. natans* are: low concentrations of nitrogen, phosphorus, dissolved oxygen, biodegradable organics, or high concentration of sulphides [4, 10]. The domination of *Sphaerotilus natans* in WWTP "Sadinata" was due to low concentration of biodegradable organics.

CONCLUSIONS

The obtained results showed that denitrification was the critical process in the technology on base of denitrification / nitrification and especially in WWTP "Sadinata". Denitrification in WWTP "Sadinata" depended on following main factors: the low concentration of biodegradable organics, the high concentration of dissolved oxygen in denitrification zone and the high pH. Denitrification/Nitrification technologies can be optimized and controlled by means of specific for the wastewater treatment plant parameters and indicators. Realization of an effective and efficacy management of the denitrification process requires the purposely elaborated design for professional control. The specific indicators for WWTP "Sadinata" include: NRA, BOD_5:COD, O_2 and pH. In WWTP "Kubratovo", the process was less influenced and the main factors that can be used for its optimization were: the concentration of nitrogen and phosphorus, in order to keep the optimal ratio among the three basic nutrients (C:N:P). The process control indicator in WWTP "Kubratovo" includes: NRA and C:N:P ratio.

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СРАВНИТЕЛНА ОЦЕНКА НА ДЕНИТРИФИКАЦИЯТА И НИТРИФИКАЦИЯТА ВЪВ ВОДОПРЕЧИСТВАТЕЛНИТЕ ТЕХНОЛОГИИ НА ПСОВ „САДИНАТА” И СПСОВ „КУБРАТОВО”

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Резюме: Технологичната схема на базата на денитрификация / нитрификация (D/N) е широко приложима при биологичното пречистване на битови и индустриални отпадъчни води, както и за инфилтрат от депа за неопасни отпадъци. Целта на настоящето изследване е да се направи сравнителна оценка между процесите на денитрификация / нитрификация в два типа пречиствателни станции и на тази база да се отдиференцират ключовите фактори за тяхното управление. Пробите за изследване са взети от биобасейните в денитрификационна и нитрификационна зона от СПСОВ „Кубратово” (за третиране на битови и промишлени отпадъчни води от гр. София) и от ПСОВ „Садината” (за третиране на инфилтрат от депото за неопасни отпадъци на гр. София). Сравнителният анализ е направен на база изследваните ключови физико-химични (pH, разтворен кислород, концентрация на органиката, азота и фосфора), технологични (C:N:P, $БПК_5$:ХПК, обемен индекс на утайката, филаментен индекс, относително обилие на филаментни микроорганизми), микробиологични (количество на аеробни хетеротрофи, денитрифициращи и нитрифициращи микроорганизми) и ензимологични (сумарна дехидрогеназна и нитратредуктазна активност) показатели.
Получените резултати показват, че в технологичната схема, включваща денитрификация / нитрификация, денитрификацията е критичен процес за водопречистването. Това особено силно важи за ПСОВ „Садината”. Като основен фактор, от който зависи процесът бе отдиференцирана концентрацията на биоразградимата органика. Установява се, че процесите на нитрификация протичат интензивно и в двете пречиствателни станции.

Ключови думи: активна утайка, денитрификация / нитрификация, микробиологичен и ензимологичен контрол, пречистване на отпадъчни води

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