Investigation of simultaneous nitrification and denitrification process using biofilm formed on intelligent hydrogel micro-carriers

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Abstract
Investigation of simultaneous nitrification and denitrification (SND) was carried out by using multiple strata proto-biofilm on PVA-PAA (Polyvinyl-alcohol-Poly-acryl-acid) copolymer in a continuously stirred, laboratory-scale reactor. The influent wastewater was originated from a large-scale wastewater treatment plant, South-Pest WWTP. The hydraulic residence time was between 8-12 h. In the course of the experiments we maintained 0.2-2 mgO_2/l dissolved oxygen level, the temperature were between 15-22 C°. The average ammonium load was 11-28 mg/h. To control the growth of heterotrophic bacteria the applied C:N ratio was in the range of 2-5; methanol as external carbon source was used. The F/M ratio was between 0.11-0.13. Average removal rate of inorganic nitrogen compounds was 53 %, being 76 % for ammonium and 50 % for nitrate and nitrite. Results show that using PVA-PAA hydrogels the optimal range of the bulk DO (0.7 - 1.5 mgO_2/l) was higher than suggested in literature due to the higher density of the biofilm and the size of the gels.

Keywords
Biofilm; hydrogel micro-carriers; nitrogen removal; simultaneous nitrification and denitrification; wastewater treatment

INTRODUCTION

In the last two decades new demand has emerged to accomplish high-efficiency nutrient (nitrogen and phosphorus) removal in municipal wastewater treatment technologies in order to reduce eutrophication processes in both inland and international watersheds. Nitrogen compounds are usually removed in biological treatment technologies, utilizing two biochemical processes: nitrification and denitrification, each requiring different environmental conditions (aerobic environment and inorganic carbon sources for nitrification versus anoxic circumstances and organic carbon sources for denitrification). In practice most often isolated anoxic and aerobic reactors are applied (pre-denitrification or post-denitrification). Therefore the investment costs (construction of new reactors and recirculation) and O&M costs (energy demand of aeration and recirculation, external carbon source, chemicals for pH control) are considerable.

To reduce the costs of biological nitrogen removal research have been conducted into the implementation of the simultaneous nitrification and denitrification (SND) (Collivignarelli et al., 1999; Zhao et al., 1999; Chiu et al., 2006). In case of SND the two processes occur in a single, aerated reactor. The SND process has important advantages: lower aeration requirement, no nitrate recirculation, less chemicals for pH control, to name a few.

There are different theories about the mechanisms of the SND; most of the studies presume the microenvironment concept. According to this theory the dissolved oxygen (DO) measured as bulk DO, does not reach the inner part of the sludge flocs, this way anoxic conditions can be reached and

denitrification can occur in the internal regions of the flocs. Previous studies have proved that there are several factors influencing SND processes, such as structure, size, density and concentration of sludge flocs, DO, F/M ratio, C/N ratio and pH, etc. (Pochana et al., 1999).

The laboratory and full-scale operational experiences have showed that efficient nitrogen removal cannot be reached by SND in the case of the conventional activated sludge system because of the low density and size of flocs (Zhang *et al.*, 2007).

In framework of the IASON (*Intelligent Activated Sludge Operated by Nanotechnology*) project (led by the Department of Sanitary and Environmental Engineering, Budapest University of Technology and Economics), we introduced a non-traditional concept by utilizing the results of nano-technological development in biological wastewater treatment process (Fleit et al., 2008). Artificially constructed micro-reactors could perform complex tasks within a single aerated basin, as the biochemical processes are regulated by the responses of the microenvironment of IASON structures. These are governed directly by the build-up of the nanostructures constructed within.

In the course of IASON project the SND was carried out by using PVA-PAA (Polyvinyl-alcohol-Poly-acryl-acid) co-polymer embedded with starch in a continuously stirred, laboratory-scale reactor using multiple strata proto-biofilm (Fleit et al., 2008). The experiences showed that multiple strata proto-biofilm cannot be kept using high C:N ratio because of the faster growth of heterotrophic microorganisms. In the present research high efficiency nitrogen removal is aimed in a continuously stirred laboratory-scale reactor by multiple strata proto-biofilm developed on PVA-PAs co-polymer embedded with starch granules based on the experiences of the IASON project.

METERIALS AND METHODS

Micro-carrier hydrogel

During the experiments PVA-PAA copolymer was used as a micro-carrier. In this carrier the regulation of the porosity (inner structure) of hydrogel pearls was achieved by the addition of fine starch suspension into the gelifying liquor hence embedding easily biodegradable substrate into the inner structure of the gels. The starch content of the gels was partially removed by a simple method (heat and chemical treatment). Removal of near-to-surface embedded starch granules from the polymer matrix provided surface roughness and micro-pores for the wastewater bacteria for initial adherence. The water content of the PVA-PAA co-polymer gel embedded starch was about 97-98 %. Hydrogel pearls have a diameter of 100-150 μm.

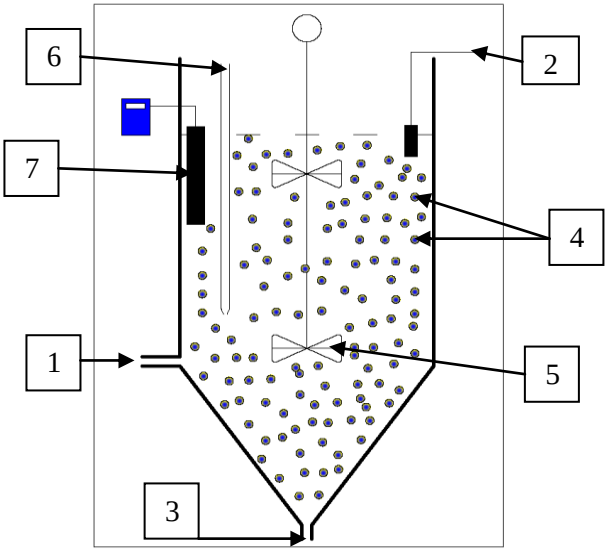
Simultaneous nitrification and denitrification

Simultaneous nitrification and denitrification was achieved by the establishment of multiple strata proto-biofilm formed by wastewater bacteria. The biofilm was attached onto the surface of the PVA-PAA hydrogel beads embedded with starch. Suspended growth activated sludge flocs or in the experiments the biofilm on hydrogel surfaces have several hundred μm in diameter. On microscopic scale this would involve the presence of steep concentration gradients. At given dissolved oxygen concentration in bulk solution anoxic or anaerobic conditions could develop the deeper layers inside the flocs or the biofilm. The upper layer of the proto-biofilm was dominated by the autotrophic nitrifying bacteria due to the suitable oxygen supply. The deeper layers of the established biofilm were composed predominantly by heterotrophic bacteria (facultative anaerobic microorganisms) hence denitrification was expected to occur in this zone. Establishment of heterogeneous layers in biofilm developing on the PVA-PAA carriers was the most difficult step in the simultaneous reactor. The structure of the biofilm depends on many environmental conditions and biotic factors

(inoculation of the gels, composition of influent (raw) wastewater, diurnal changes of influent wastewater, flow conditions and apparent shear forces in the reactor, the continuously changing density and average thickness of the biofilm, texture and 3D structure of the biofilm). Numerous papers report various effects of presence or absence of microscopic channels and voids, dosing of the methanol, buffer solutions on biofilm structure (Chang et al., 1991; Klapper et al., 2002). The texture of the double layers biofilm could be controlled indirectly by the concentration levels of various electron acceptors and donors in influent wastewater (such as NH_4-N, NO_2-N, NO_3-N) and aeration intensity. Environmental conditions in bulk solution (i.e. the reactor space) can be finely tuned by regulating intensity of aeration (fine and coarse bubble aeration), the methanol dosing that indirectly affects dissolved oxygen concentration, etc. Conditions favouring the growth of heterotrophic biomass having a higher growth rate than autotrophic counterparts could be altered by organic carbon source (methanol) dosage as evidenced by microscopic observation during the experiments.

Laboratory scale experimental system

The outline of the laboratory scale experimental system for simultaneous nitrification and denitrification is shown on Figure 1. The system consists of one continuously stirred and aerated reactor, (hydraulic volume 4.5-6.3 l). Methanol dosage was regulated by the nitrate (40-70 mg/l) dissolved oxygen concentration measured at sampling point 2 on Figure 1.



Hydraulic reactor volume:	5-7 l
Volume of the PVA-PAA gels:	500 ml
Hydraulic loading:	0.4-0.6 l/h
Methanol dosing:	9.88-39.5 mg/l
(Corresponding to 80-320 mg/l COD value)	

Figure 1. The laboratory-scale experiment system: (1) Feed water, methanol dosing, (2) effluent, (3) Aeration, (4) Hydrogels, (5) Stirrer, (6) Inoculation, (7) DO probe

Colonization process

The colonization process and the inoculation technique were developed in the course of IASON project (Fleit et al., 2008). The inoculation bacterial suspension was made from different type of bacteria. In case of heterotrophic denitrifying biofilm the basic bacterial suspension was the cleaning water of the denitrifying biofilter of a communal wastewater treatment plant (South-Pest WWTP) and the basic suspension of the autotrophic bacterial suspension was the cleaning water of the nitrifying biofilter of the same plant. Wastewater of the effluent of secondary clarifiers from the South-Pest WWTP was utilized as feed water to the system. Table 1. shows the characteristic values of the feed water (nitrogen compounds can be seen in Table 2. and Figure 5. and 8.).

Table 1. Influent wastewater characteristics.

Parameter	Average	Minimum	Maximum
pH	7.78	7.43	8.12
Conductivity μS/cm	1862	1761	1924
o-PO_4-P mg/l	1.6	0.16	3.2
SS mg/l	5.5	4.2	6
COD mg/l	95	81	119

Most of the carbon compounds were removed from the wastewater in the WWTP, the ammonium was removed in the laboratory in a nitrifying SBR reactor, provided the high concentration of nitrate in feed water (Table 2.). In the course of the colonization and SND experiments NH_4Cl and Na_2CaCO_3 were added to the feed water to ensure the necessary ammonium concentration and inorganic carbon source for nitrification.

The preparation techniques of the inoculated suspension were the same in case of both types of the biofilm. Prior to inoculation the suspended biomass was treated by ultrasonic treatment for disaggregating the flocs followed by a filtration step on 50 μm glass fibre filter to avoid any spontaneous flocculation phenomena. To avoid the high concentration of the bacterial cells and flocs in the reactor the bacterial suspension was dosed slowly for 24 hours at the reactor start-up period. The quantity of the inoculated suspension was optimized according to the amount of the gel beads in the reactor and the size of the reactor. A multiple layer biofilm was established on the surface of the gels by double step inoculation process. First suspension of heterotrophic bacterial cells was dosed to the reactor twice at 24 h intervals. In the second step of inoculation autotrophic nitrifying bacteria were dosed into the reactor on the 12^{th} day of the inoculation process. Different types of feed water were used in the colonization process of the two layers. Table 2. shows the characteristic values of the nitrogen compounds in the feed water during the colonization.

Table 2. The concentration of nitrogen compounds in feed water during the colonization.

		Colonization of heterotrophic layer			**Colonization of autotrophic layer**		
Parameter	Unit	Minimum	Maximum	Average	Minimum	Maximum	Average
Total N	mg/l	39	71	45	35	56	42
NH_4-N	mg/l	0	0	0	23.8	52.2	30
NO_2-N	mg/l	0.2	3.3	1.2	0.07	0.9	0.19
NO_3-N	mg/l	37	67	43	34	54	41

In order to establish the nitrifying layer DO level was kept above 2.0 mgO_2/l, and less methanol (80 mgCOD/l) was added to the feed water. The presence of spontaneously suspended flocs (truly "planktonic" biomass portion) in the bioreactor might cause false results as well as structural damage in biofilm. Removal of spontaneously formed suspended planktonic flocs from the bioreactor was maintained on a continuous basis complemented by thorough washing and withdrawal of the not gel-bound biomass at every second day. Environmental conditions such as

temperature, exposure time, concentration of suspended bacteria (initial inoculum concentration) and most notably reactor hydraulics affected bacterial adhesion and the ultimate structure of formed biofilm.

The Figure 3a-3d. shows the development of the biofilm on the surfaces of the gels beads.

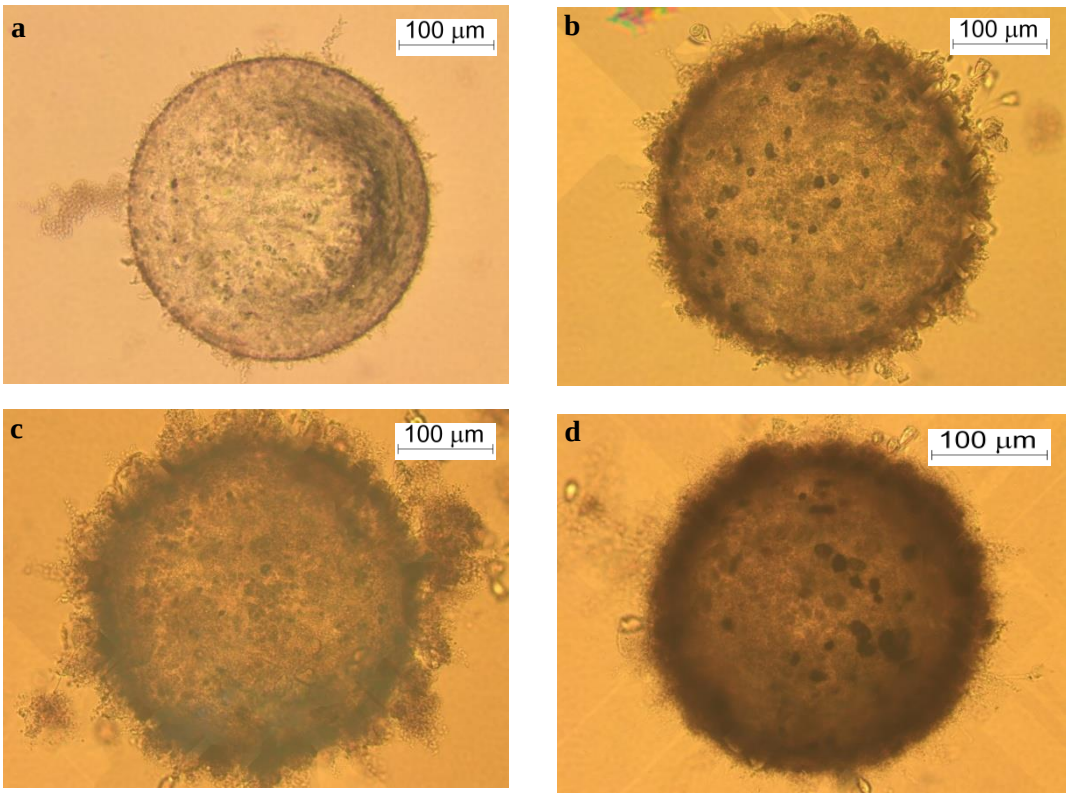


Figure 3. Biofilm development on NaOH treated starch containing PVA–PAA hydrogel beads. **a)** State of heterotrophic biofilm development on Day 8. At this time the second stage of the inoculation was started. **b)** Multiple layered biofilm on Day 47, *Epistylis* spp. and *Vorticella* spp. started to grow on the surface of the autotrophic biofilm indicating non-toxic environmental conditions and high sludge retention time. **c)** Heterotrophic biomass from deeper biofilm layer starts to overgrow the upper autotrophic biofilm. **d)** Fully developed multiple layer mature biofilm on Day 73.

After Day 38 the multilayer biofilms were developed. During the colonization process the activity of nitrifying and denitrifying bacteria were measured. Figure 4. shows the change of ammonium, nitrite and nitrate concentration.

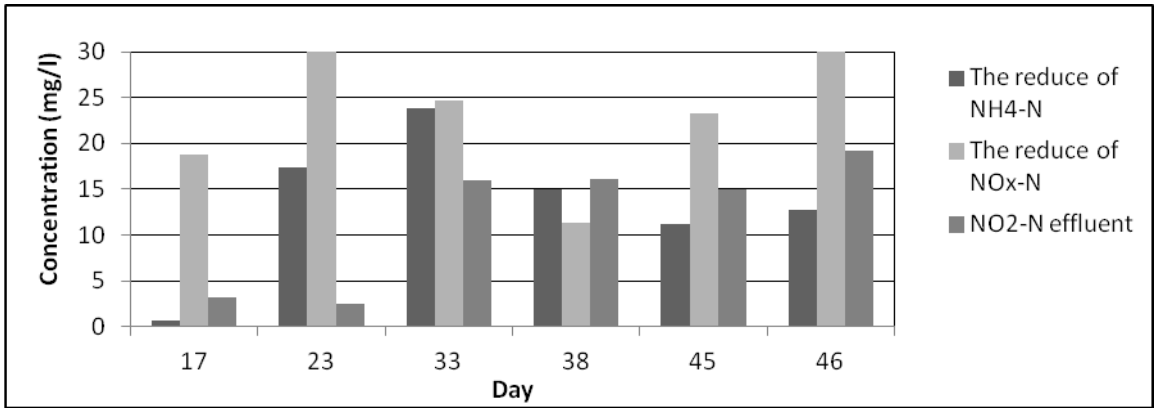


Figure 4. The activation of nitrification and denitrification during colonization.

Figure 4. shows that after Day 23 the activity of nitrifying bacteria became significant; ammonium was reduced by 24 mgNH_4-N/l. After Day 38 lower DO level (<2.0 mgO_2/l) and higher methanol dose (160 mg/l) were used in order to increase the activation of denitrification.

SIMULTANEOUS NITRIFICATION AND DENITRIFICATION EXPERIMENTS

After the colonization detailed investigation of SND process was commenced. High nitrate and ammonium load were used in the feed water. The DO concentrations were in the range of 0.2-2.0 mgO_2/l. The HRT was 8-12 hours in the reactor. The MLVSS concentration varied between 1.7-2.0 g/l. Figure 5. shows ammonium and nitrate concentration of the influent wastewater.

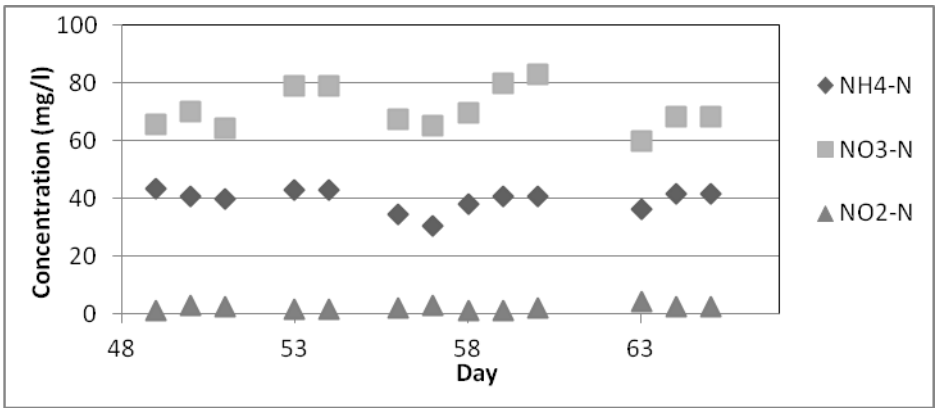


Figure 5. Inorganic nitrogen forms in feed water during Day 49-65.

To control the process of SND methanol dose and DO level were optimised to reach efficient nitrification and denitrification. To raise the efficiency of denitrification higher methanol dose were used (320 mgCOD/l) between Day 49-51. Denitrification activity increased (Figure 6.), parallel to the reduction of nitrification efficiency and heterotrophic biomass from deeper biofilm layer started to overgrow the upper autotrophic biofilm (Figure 3c).

To sustain the autotrophic layer after Day 51 less methanol (160 mgCOD/l) were used (and kept constant afterword). According to the microscopic investigation the multiply biofilm layers could be sustained among these conditions (see Figure 3d.).

The effect of DO level on SND process were investigated after stabilising the multiply biofilm layers. Results of the measurements between Day 49-65 can be seen on Figure 6.

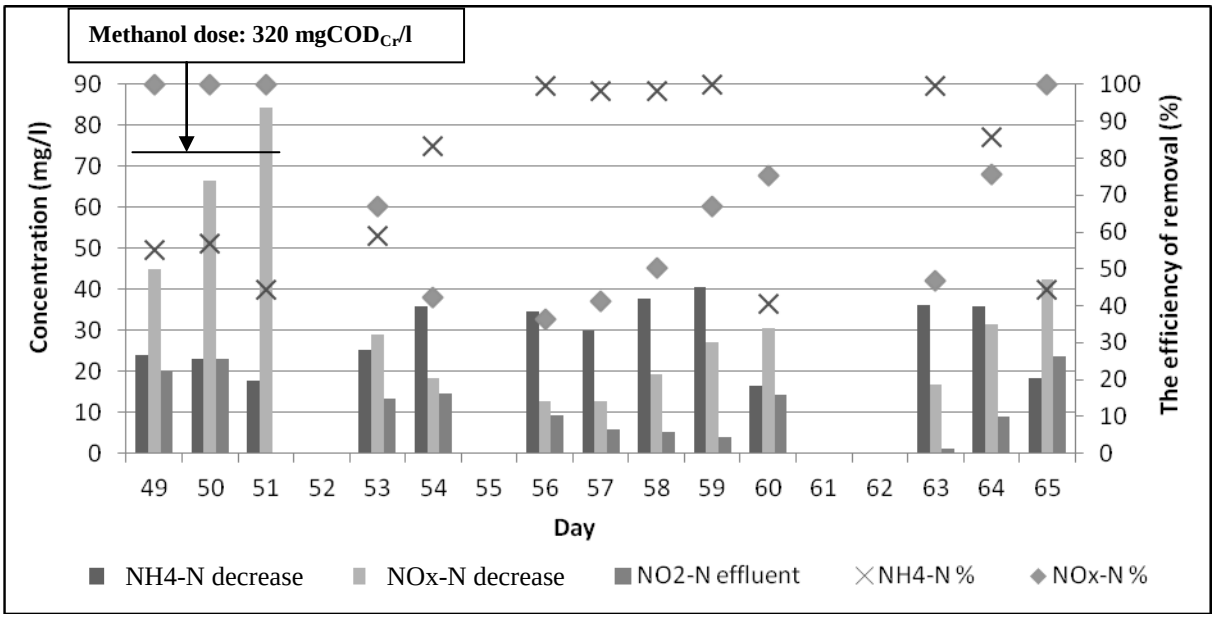


Figure 6. The results of NH_4-N, NO_2-N and NO_3-N measurements during Day 49-65 (the initial NH_4-N concentration was considered as 100 %).

Figure 7. shows how different DO level changed the efficiency of SND process.

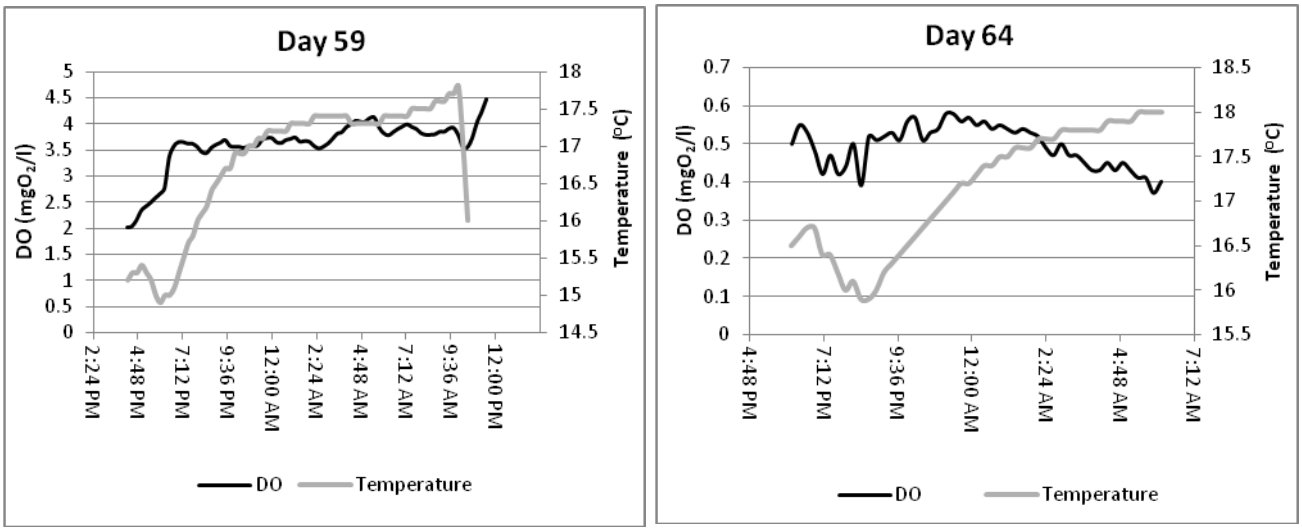


Figure 7. DO level and temperature on Day 59 and 64.

On Day 59 the nitrification was complete, and the efficiency of denitrification was 67 % in spite of the high DO level (>2 mgO_2/l). This phenomenon proves that oxygen gradient formed in the biofilm layer. On Day 64 DO level was between 0.4-0.6 mgO_2/l, which is the ideal range for SND according to earlier studies (Zhang et al., 2007). In this range nitrification was not complete and nitrite appeared in the effluent as well, but the denitrification was more efficient the on Day 64.

In the second stage of the SND experiment different feed water was used with lower nitrate concentration (see Figure 8.).

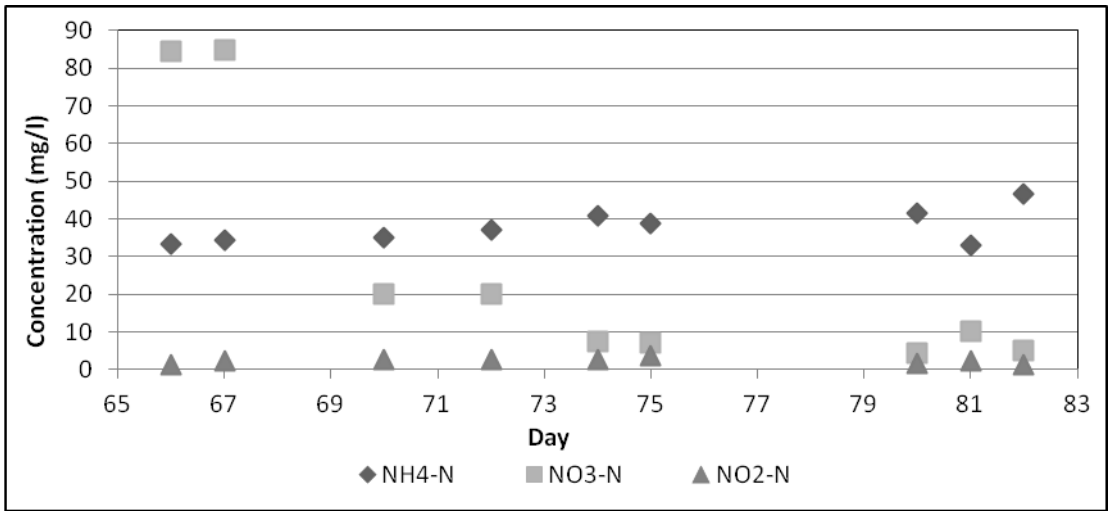


Figure 8. The inorganic nitrogen forms in feed water during Day 66-82.

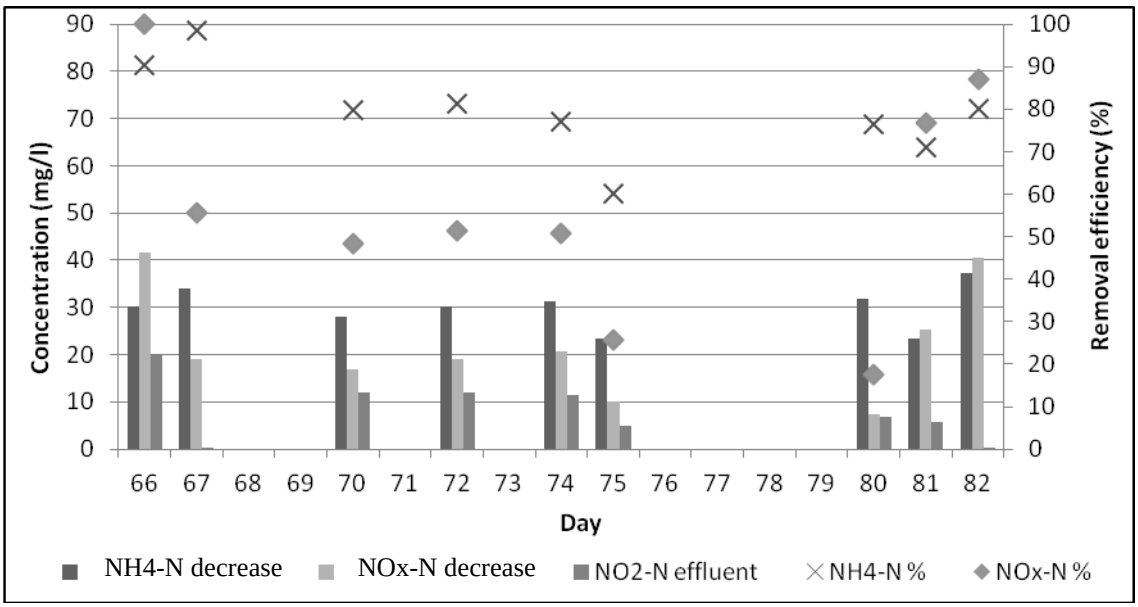
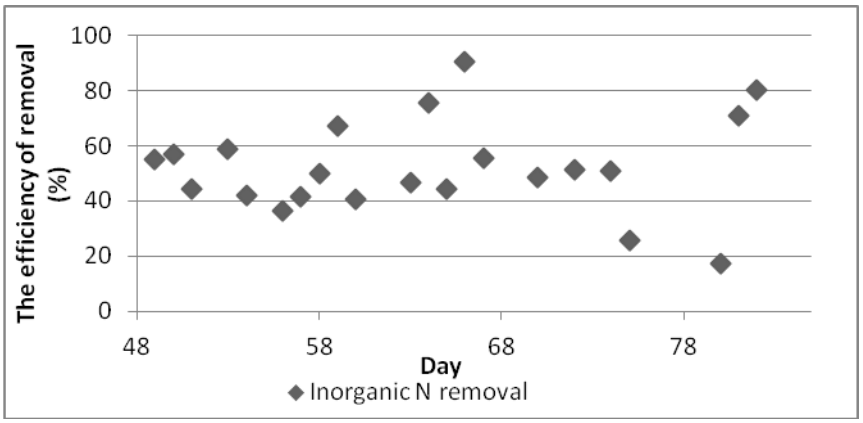


Figure 9. The results of NH_4-N, NO_2-N and NO_3-N measurements during Day 66-82 (initial NH_4-N concentration was considered as 100 %).

According to the results nitrification and denitrification were efficient in the case of lower initial nitrate concentration as well. The removal efficiency of ammonium was above 70 % and above 50 % of NO_X-N in most cases. Significant nitrite concentration showed up in the effluent, which proved that denitrification can be achieved via nitrite instead of nitrate. Effluent nitrite and nitrate concentration was in similar range (5-10 mg/l). Several studies (Yoo et al., 1999) mentioned the short-cut denitrification, when the Nitrobacter is inhibited, therefore nitration does not happen and the denitrifying bacteria use nitrite as electron acceptor. Inhibition can be related to DO, pH, free ammonia concentration, low C/N ratio according to the earlier studies (Yoo et al., 1999). Alkalinity of the influent wastewater was high; therefore the reason probably could be the DO level and the low C/N ratio in this case. The MLVSS concentration was 1.8-2.1 g/l in the reactor.



Day	C/N
57	2.5
58	2.4
66	2.3
67	2.0
70	4.6
72	4.4
74	5.1
75	5.1
80	5.0
81	5.3

Figure 10. Inorganic nitrogen removal efficiency and C/N ratio (initial NH_4-N ratio was considered as TN in the influent wastewater).

Figure 10. shows that inorganic nitrogen removal fluctuated at around 50 %. Average removal rate was 52 %. According to previous experiences (Collivignarelli et al., 1999) similar efficiency can be achieved with pre-denitrification system in activated sludge treatment. The C/N ratio varied between 2.0-5.3; according to the literature C/N≥7 is necessary for denitrification (Barnard et al., 1992).

CONCLUSIONS

In the course of the experiments SND was investigated using multiple strata proto-biofilm which was developed and maintained during 1.5 month after the inoculation phase. To control the growth of heterotrophic bacteria the applied C:N ratio varied between 2-5, which was smaller than the optimal value according to the literature. Average removal rate of inorganic nitrogen compounds was 53 % with ammonium removal of 76 % and nitrate and nitrite removal of 50 %. According to the literature the optimal range of dissolved bulk oxygen concentration for SND is about 0.3-0.8 mgO_2/l regarding to the limitations of oxygen diffusion into the flocs. Using PVA-PAs hydrogels the optimal range of the bulk DO was between 0.7 and 1.5 mgO_2/l, in this range the inorganic nitrogen removal reached the 80 %; the explanation of these differences can be related with the higher density of the biofilm and the size of the gels. In the effluent high concentration of nitrite was observed in some cases because of the insufficient oxygen conditions. Some studies show that denitrification can carried out via nitrite instead of nitrate. According to the results of the presented experiments it is unambiguous that heterotrophic microorganism used nitrite, because both nitrite and nitrate were detected in the effluent. This phenomenon requires further studies to examine the denitrification process via nitrite.

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