

Nitrogen transformations and mass balances in anaerobic/anoxic/aerobic batch experiments with full-scale biomasses from BNR activated sludge systems

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ABSTRACT

The aim of this study was to investigate nitrogen mass balances occurring inside full-scale BNR activated sludge systems, with special attention to colloidal and dissolved organic nitrogen (CON and DON) transformations. For this purpose, laboratory experiments were carried out using process biomass from two large BNR plants in northern Poland. Two parallel batch reactors were operated in a 3-phase (anaerobic/anoxic/aerobic) cycle. In one reactor, the settled wastewater without any pretreatment was used, whereas the settled wastewater after coagulation-flocculation (to remove colloidal and particulate fractions) was added to another reactor. The chemical pretreatment of settled wastewater with $ZnSO_4$ did not adversely affect the observed nitrification rates in the (last) aerobic phase. It caused, however, a reduction of denitrification rates in the anoxic phase. Moreover, the chemical pretreatment did not appear to generally decrease DON but decreased CON. DON was explicitly produced in the aerobic phase and organic nitrogen conversion also occurred at a significant rate in the anoxic phase with biodegradable COD consumption and solids hydrolysis. The inorganic N mass balances revealed N losses up to approximately 10% which could be attributed to a few novel pathways of nitrogen removal, most likely aerobic denitrification or simultaneous nitrification/denitrification.

Key words | batch test, denitrification, dissolved organic nitrogen, nitrification, nitrogen removal

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INTRODUCTION

In municipal wastewater treatment plants (WWTPs), influent total nitrogen (TN), consisting of ammonia/ammonium (NH_4-N) and particulate (PON), colloidal (CON) and dissolved (DON) organic nitrogen may follow a number of transformation pathways (Figure 1) leading to an effluent total nitrogen composed of the following constituents:

$$TN_{\text{eff}} = NH_4-N + NO_X-N + PON + CON + DON \quad (1)$$

Nitrogen removal processes in biological nutrient removal (BNR) WWTPs involve nitrogen removed for biomass synthesis and sludge wasting, nitrification/

denitrification to remove inorganic N fractions (ammonia/ammonium (NH_4-N) and nitrate/nitrite (NO_X-N)), and solid-liquid separation to remove PON and CON fractions. For BNR systems designed to maximize nitrification/denitrification and effluent solids removal via filtration of clarifier effluent or use of membrane bioreactors (MBRs), the TN_{eff} concentration may range from 2.0 to 4.0 mg/L with about 40% as effluent organic nitrogen (EON); mainly as CON and DON (Stensel *et al.* 2008). In this case DON is defined as filtrate organic nitrogen passing a 0.10- μm filter and CON is from filtrate between 1.2- and 0.10- μm filtration. More recently, with lower TN_{eff} goals in Europe and the United States, the contribution of EON has become very important. Present knowledge on the characteristics

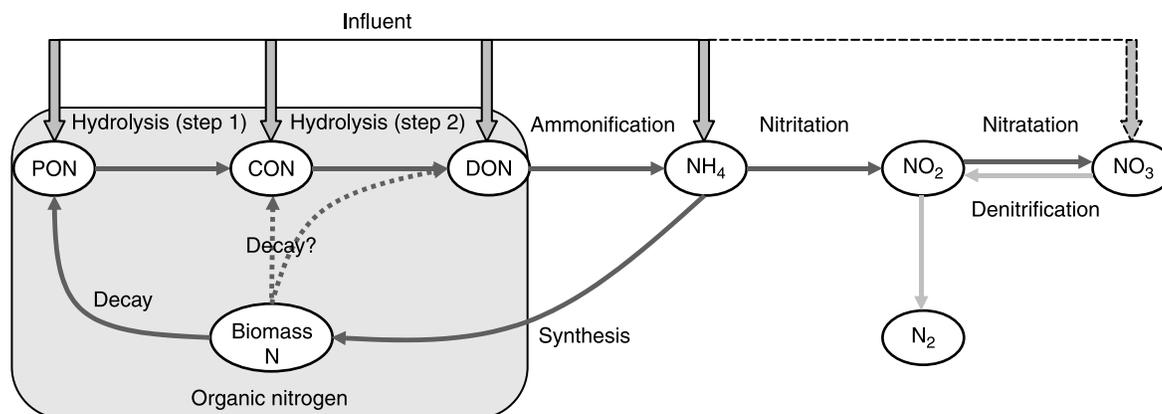


Figure 1 | Diagram showing possible N transformation pathways in activated sludge bioreactors.

and behaviour of CON and DON is limited and insufficient to estimate BNR process effluent concentrations as a function of plant design and influent characteristics.

Very little has been done on the fate of CON and DON in WWTPs since the early work of *Parkin & McCarty (1981)* on the fate and characteristics of filtered effluent organic nitrogen (CON + DON) at the Palo Alto, California (USA) WWTP. The average filtered EON concentration was approximately 1.5 gN/m^3 . Based on the N mass balance, the authors found that 52% of it was recalcitrant (resistant to biological transformations) from influent wastewater sources, 20% was produced from biomass endogenous decay in the activated sludge process, 15% was in equilibrium between that sorbed to biomass and the liquid and about 13% could be further degraded. Furthermore, the authors noted that increasing the activated sludge SRT could further degrade influent filtered organic N, but it could also be added via biomass endogenous respiration.

Studies in several WWTPs in the US and Poland suggested that effluent CON and DON concentrations are independent of the influent TN concentrations and process configurations (*Pagilla et al. 2008*). However, one of the potential methods posed to enhance DON and CON removal is to increase the SRT in activated sludge systems (*Pagilla et al. 2006*). However, there are no reported studies confirming this hypothesis. *Dignac et al. (2000)* found that the biological nutrient removal (BNR) processes can be efficient in removing low molecular weight (LMW) organic matter and DON compounds such as urea, amino acids, small nitrogen containing organic molecules and

proteins. In contrast, the high molecular weight (HMW) substances may not be removed using the common activated sludge process.

Stensel et al. (2008) emphasized the need for research to determine what design and operating conditions for a BNR facility affect the biodegradable and non-biodegradable fractions of effluent DON. The authors raised several key issues for this research including the following questions: how is DON removed or produced in BNR processes and is there an effect of the BNR design and configuration (anaerobic and anoxic contact) on effluent DON? This study was in line with these research needs. Its aim was to investigate nitrogen mass balances occurring inside full-scale BNR activated sludge systems, with special attention to CON and DON transformations. For this purpose, laboratory batch experiments were carried out using process biomass from two large BNR plants in northern Poland.

METHODS

Process configuration and performance of the studied plants

Experimental studies were carried out at the plants located in two neighbouring cities of Gdansk (approx. 500,000 inhabitants) and Gdynia (approx. 330,000 inhabitants) in northern Poland. Both plants discharge the treated wastewater to the Bay of Gdansk which is part of the Baltic Sea.

Wschod WWTP

The “Wschod” WWTP (approx. 570,000 PE) in Gdansk is one of the largest facilities located upon the Baltic Sea. The biological step consists of six parallel bioreactors and twelve circular secondary clarifiers. The bioreactors run in the MUCT (modified University of Cape Town) process configuration with an additional deoxic zone in the internal recirculation lane from the aerobic zone to the anoxic zone. The volume of a single bioreactor is 26,350 m³.

Debogorze WWTP

The “Debogorze” WWTP (approx. 515,000 PE) in Gdynia treats wastewater originating from the city of Gdynia and four surrounding smaller towns. The existing biological step, run in the Johannesburg (JHB) process configuration, consists of four parallel bioreactors and six secondary clarifiers. The volume of a single bioreactor is 12,000 m³. The plant is being expanded now with four new JHB reactors.

The basic characteristics of both studied WWTPs are presented in Table 1. In the study of Czerwionka *et al.* (2009), the influent and effluent organic N fractions were investigated in more detail during ten sampling campaigns between March, 2007 and December, 2008. The average influent TON concentrations were very similar at the two plants, i.e. 27.9 gN/m³ (± 7.6 gN/m³) in Gdansk vs. 28.8 gN/m³ (± 4.9 gN/m³) in Gdynia. The contributions of DON and CON were 6% and 28% (Gdansk), and 8% and 34% (Gdynia). With regard to the effluent TON, its concentrations were 2.6 (± 0.57) and 3.4 gN/m³ (± 1.51 gN/m³), respectively, in Gdansk and Gdynia. The contributions of “truly” DON and CON increased to 28% and 34% (Gdansk), and 26% and 38% (Gdynia).

Procedures of lab-scale experiments

Lab experiments were carried out in an experimental set-up consisting of two parallel batch reactors (max. volume of 4.0 dm³), control system and computer. The reactors were equipped with electrodes for a continuous monitoring of pH, ORP, temperature and dissolved oxygen (DO). The automated control system maintained a desired DO concentration and temperature in the reactors. This system

Table 1 | Characteristics of the studied WWTPs in Gdansk and Gdynia (average values for the period 2007–2008)

Parameter	Unit	WWTP	
		Gdansk	Gdynia
Process configuration	–	MUCT	JHB
Size	PE	570,000	515,000
Influent flowrate	m ³ /d	81,000	56,000
<i>Influent characteristics</i>			
COD	gCOD/m ³	1,007	1,091
“Soluble” COD	gCOD/m ³	278	–
N _{tot.}	gN/m ³	83.8	82.5
N-NH ₄ ⁺	gN/m ³	58.2	57.7
P _{tot.}	gP/m ³	13.2	11.1
<i>Operating parameters</i>			
SRT	d	19	18
MLSS		5.3	5.1
<i>Effluent characteristics</i>			
COD	gCOD/m ³	48.6	39.1
N _{tot.}	gN/m ³	11.8	12.9
N-NH ₄ ⁺	gN/m ³	1.4	1.9
N-NO ₃ ⁻	gN/m ³	7.5	8.2
P _{tot.}	gP/m ³	0.63	0.82

also controlled a continuous measurement (in a cyclic mode) of oxygen uptake rate (OUR) in a small chamber connected to the main unit.

During the batch experiments, both reactors were operated in a 3-phase cycle including anaerobic conditions (2 h), anoxic conditions (4 h, after addition of KNO₃) and aerobic conditions (6 h). In reactor 1, the process biomass (fresh returned activated sludge) was diluted with the settled wastewater without any pretreatment (containing soluble, colloidal and particulate organic fractions), whereas the settled wastewater after coagulation-flocculation was mixed with the process biomass in reactor 2 (Figure 2). The latter sample of wastewater, only containing a soluble organic fraction, was prepared according to the rapid physical-chemical method of Mamais *et al.* (1993) based on precipitation with ZnSO₄ at pH = 10.5. After removing colloids and particulates, the pH was adjusted to its original value by adding 6 M HCl.

The dilution rate of process biomass was adjusted to obtain MLSS at approx. 2.5 kg/m³ in the reactors. After adding the mixture to the reactor, the mixer was turned on.

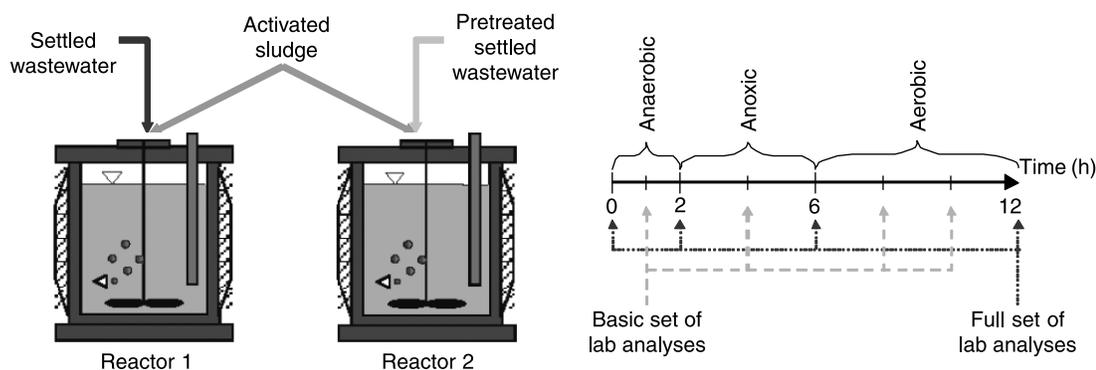


Figure 2 | Diagram showing the experimental procedure with two parallel batch reactors, sampling schedule and scope of the lab analyses.

The actual MLSS concentrations were measured at both beginning and end of the experiment. Samples of 100–150 cm³ were withdrawn with the frequency shown in Figure 2, filtered under vacuum pressure on 1.2 µm pore size filter and then analysed. The “basic” set of lab analyses included NH₄-N, NO₂-N, NO₃-N, COD and PO₄-P, whereas the “full” set of lab analyses was extended with additional TN and TOC measurements in 1.2, 0.45 and 0.1 µm pore size filtrates. At the beginning of the anoxic phase (2 hour), potassium nitrate (KNO₃) in order to rise the initial concentration of NO₃-N by 20 gN/m³. At the beginning of the aerobic phase (6 hour), the aeration system was turned on and the dissolved oxygen (DO) set point was controlled at 6 g O₂/m³ during the test. The automatic OUR measurements were also initiated.

Analytical methods

The samples were filtered through membrane filters of different pore sizes including 0.1, 0.45 and 1.2 µm pore size Millipore nitrocellulose filters (Billerica, MA). Then the filtrate was analyzed for TN using TOC/TN analyzer (Shimadzu corporation, Japan) and inorganic forms of nitrogen (NH₄-N, NO₃-N and NO₂-N) using Xion 500 spectrophotometer (Dr Lange GmbH, Germany). The analytical procedures, which were adapted by Dr Lange GmbH (Germany) and Shimadzu (Japan), followed the Standard Methods (APHA 1992). The DON and CON concentrations were estimated from the difference between TN after filtration and the sum of inorganic N concentrations.

RESULTS AND DISCUSSION

Principal nitrogen transformations and mass balances in the batch reactors

The behaviour of all inorganic N forms (NH₄-N, NO₃-N, NO₂-N) was very consistent in the tests with process biomass from both studied plants. The examples of this behaviour are shown in Figure 3 (Gdansk) and Figure 4 (Gdynia), and the observed utilization/production rates are summarized in Table 2.

The denitrification rates (NURs) listed in Table 2 are primarily associated with utilization of the slowly biodegradable substrate (and/or stored intracellular polymers) as most of the readily biodegradable substrates were used in the preceding anaerobic phase. It is apparent that chemical precipitation affected the measured NURs and particulate organic compounds played an important role in enhancing denitrification. This finding was confirmed by the fact that the rates in reactor 1 (containing soluble, colloidal and particulate organic fractions) were higher up to approximately 25% (Gdansk) and 30% (Gdynia) compared to the parallel reactor 2 (containing only soluble organic fractions).

In the earlier study with the settled wastewater without pretreatment (Makinia 2006), the denitrification rates were measured in similar experiments consisting of two phases: anaerobic and anoxic. The NUR values were higher (at higher temperatures) compared to this study and varied within similar ranges at both studied plants, i.e. 2.5–3.3 gN/(kgVSS h) at $T = 15.4\text{--}18.7^\circ\text{C}$ and

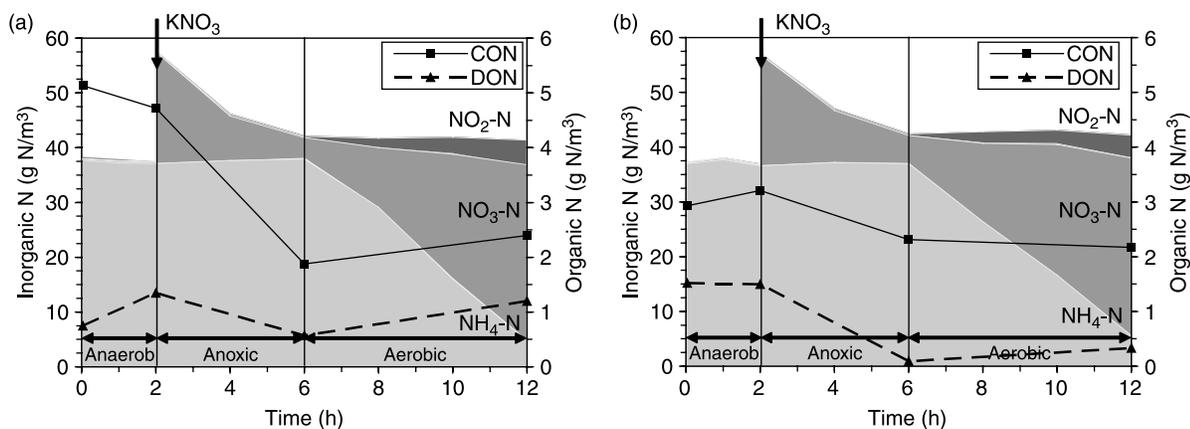


Figure 3 | Example of the three-phase batch experiment with process biomass at the Gdansk WWTP: (a) addition of the settled wastewater without pretreatment (MLVSS = 2.82 kg/m³, $T = 13.2^{\circ}\text{C}$), (b) addition of the settled wastewater after coagulation/flocculation (MLVSS = 2.82 kg/m³, $T = 13.2^{\circ}\text{C}$).

2.1–3.7 gN/(kgVSS h) at $T = 17.7$ – 20.1°C , respectively, in Gdansk and Gdynia.

The nitrification rates, expressed as ammonia utilization rates (AURs), were slightly higher at the Gdansk WWTP compared to the Gdynia WWTP. At both plants, however, hardly any differences in the process kinetics were observed in reactor 1 and reactor 2. This suggests that chemical precipitation and removal of colloidal and particulate fractions had no (or minor) effect on nitrification. It should be noted that a complete ammonia oxidation was not achieved in the batch reactors and $\text{NO}_2\text{-N}$ was accumulated reaching even the level of 8–9 gN/m³ in one test at the Gdansk WWTP. During the other tests, the final $\text{NO}_2\text{-N}$ concentrations ranged from 2 to 4 gN/m³. In contrast, no $\text{NO}_2\text{-N}$ accumulation was observed during

the measurements in the aerobic zones of the full-scale bioreactors (Czerwionka et al. 2008) nor in the secondary effluents during routine samplings by the plant operators.

The nitrification rates listed in Table 2 are consistent with the results of an earlier study (Makinia et al. 2006) when the nitrification rates were measured at higher temperatures. In that study, the specific AURs at the Gdansk WWTP ranged from 3.3 gN/(kgVSS h) at $T = 15.8^{\circ}\text{C}$ to 5.4 gN/(kgVSS h) at $T = 19.2^{\circ}\text{C}$, whereas the corresponding rates of $\text{NO}_3\text{-N}$ production were lower than the corresponding AURs by approximately 11–12%. At the Gdynia WWTP, the lowest specific (AUR = 2.0 gN/(kgVSS h) at $T = 20.3^{\circ}\text{C}$) was measured during the experimental series in the period May–June when the nitrification capacity was not fully recovered after the winter nitrification loss.

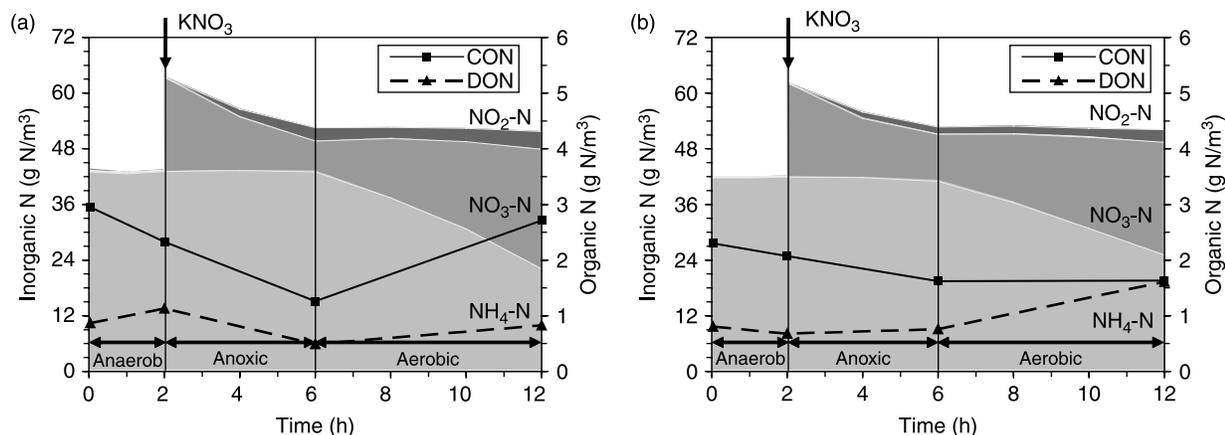


Figure 4 | Example of the three-phase batch experiment with process biomass at the Gdynia WWTP: (a) addition of the settled wastewater without pretreatment (MLVSS = 1.68 kg/m³, $T = 13.1^{\circ}\text{C}$), (b) addition of the settled wastewater after coagulation/flocculation (MLVSS = 1.38 kg/m³, $T = 13.1^{\circ}\text{C}$).

Table 2 | Summary of the rates observed during the three-phase batch experiments with process biomass at the studied WWTPs

Phase/process	Unit	Gdansk WWTP		Gdynia WWTP	
		Reactor 1	Reactor 2	Reactor 1	Reactor 2
		(T = 12.8–16.6°C)		(T = 13.1–16.6°C)	
<i>Anoxic</i>					
NUR	gN/(kgVSS h)	1.5–1.7	1.2–1.6	1.4–2.0	1.2–1.8
<i>Aerobic</i>					
AUR	gN/(kgVSS h)	2.0–2.8	1.8–3.2	1.8–2.2	1.8–2.2
NO ₃ -N production rate	gN/(kgVSS h)	1.7–2.0	1.6–2.0	1.5–2.0	1.5–1.7
NO ₂ -N production rate	gN/(kgVSS h)	0.12–0.75	0.17–0.94	0.06–0.18	0.13–0.26

The other six AUR measurements varied within the range 3.0–4.9 gN/(kgVSS h), at $T = 17.1–21.6$ °C. In this case, the rates of NO₃-N production were lower than the corresponding AURs by only 2.6–6.7%. The different specific nitrification rates at both studied plants resulted in estimating different values of the maximum specific growth of nitrifiers at $T = 20$ °C, $\mu_{A,max}$, i.e. 1.2 d^{-1} (Gdansk) and 1.0 d^{-1} (Gdynia).

Based on the conceptual model presented in Figure 1, the inorganic N mass balances were calculated for the aerobic phase. At the Gdansk WWTP, 0.7–1.2 gN/m³ and 0.2–2.6 gN/m³, respectively, were lost in reactor 1 and reactor 2. The corresponding losses in the experiments at the Gdynia WWTP were 0.7–2.2 gN/m³ (reactor 1) and 0.6–1.8 gN/m³ (reactor 2). These losses resulted in considerable N mass imbalances (up to 9% in Gdansk and 11% in Gdynia) which could be attributed to a few novel pathways of nitrogen removal; aerobic denitrification or simultaneous nitrification/denitrification (SNdN). Literature data are not consistent regarding this phenomenon. For example, Littleton *et al.* (2003) did not find substantial contributions to nitrogen removal by novel processes including autotrophic denitrification and heterotrophic nitrification-aerobic denitrification for nitrogen removal in three BNR activated sludge systems. On the other hand, Oh & Silverstein (1999) claimed that aerobic denitrification could be persistent in activated sludge systems. The authors investigated the effect of dissolved oxygen (DO), over a range of 0.09 to 5.6 g O₂/m³, on denitrification by activated sludge in a bench scale SBR. Even the DO concentration as low as 0.09 g O₂/m³ was found to inhibit denitrification, resulting in a rate decrease of 35% compared to the “true”

anoxic conditions. On the other hand, some denitrification activity (4% of the “true” anoxic rate) was observed with the DO levels as high as 5.6 g O₂/m³.

CON and DON transformations in the batch reactors

A different behaviour of CON and DON was observed during the batch experiments (Figures 3 and 4). The average measured CON and DON concentrations for all the experiments are presented in Table 3. In all the cases, the DON was explicitly produced in the last (aerobic) phase. At the Gdansk plant, its concentrations increased in the ranges 0.6–1.9 gN/m³ and 0.2–1.3 gN/m³ in reactor 1 and reactor 2, respectively, whereas the corresponding increases at the Gdynia plant were 0.3–1.1 gN/m³ (reactor 1) and 0.9–1.6 gN/m³ (reactor 2). These findings are in contradiction to the observations in the full-scale bioreactors where the concentrations of DON along the bioreactor did not change significantly (Czerwionka *et al.* 2008). However, they may reflect the production of DON with more aeration time and cell lysis as posed by Parkin & McCarty (1981).

During the batch experiments at the Gdansk plant, an apparent production of DON from CON hydrolysis occurred in the anoxic phase. This finding was derived based on lower amounts of CON utilized and higher amounts of DON produced in reactor 2 (containing exclusively the soluble fraction) compared to reactor 1 (containing all the fractions). These results are consistent with the full-scale measurements. In the anoxic compartment of the bioreactor, the concentrations of “low” (0.1–0.45 μm) and “high” (0.45–1.2 μm) colloidal subfractions decreased by 5.5 gN/m³ and 4.5 gN/m³, respectively,

Table 3 | CON and DON concentrations (g N/m^3) during the batch experiments

Sample	Reactor 1 (without pretreatment)			Reactor 2 (with pretreatment)		
	DON	CON	Total	DON	CON	Total
<i>Gdansk (average \pm standard deviations from 3 experiments)</i>						
Start	0.5 ± 0.24	3.2 ± 1.85	3.7 ± 1.08	0.7 ± 0.68	2.0 ± 1.19	2.7 ± 1.66
Anaerobic	0.6 ± 0.64	3.4 ± 1.38	4.0 ± 1.92	0.9 ± 0.57	2.1 ± 1.17	3.0 ± 1.60
Anoxic	0.4 ± 0.14	1.9 ± 0.41	2.3 ± 0.34	0.2 ± 0.11	2.2 ± 1.22	2.4 ± 1.27
Aerobic	1.5 ± 0.76	1.9 ± 0.98	3.4 ± 0.24	0.9 ± 0.58	1.9 ± 1.33	2.8 ± 1.09
<i>Gdynia (average \pm standard deviations from 4 experiments)</i>						
Start	0.5 ± 0.16	2.3 ± 1.07	2.8 ± 1.12	0.6 ± 0.28	1.7 ± 0.89	2.3 ± 0.78
Anaerobic	0.6 ± 0.39	1.9 ± 0.88	2.5 ± 1.12	0.6 ± 0.14	1.6 ± 0.56	2.2 ± 0.70
Anoxic	0.5 ± 0.16	1.4 ± 0.40	1.8 ± 0.55	0.7 ± 0.07	1.7 ± 0.81	2.4 ± 0.87
Aerobic	1.0 ± 0.63	2.0 ± 0.73	3.0 ± 1.23	1.6 ± 0.76	1.9 ± 1.11	3.5 ± 1.66

and these were the largest reductions of the colloidal subfractions observed in the entire bioreactor. It should be noted, however, that other mechanisms of CON removal are also possible and include a direct hydrolysis to ammonia and adsorption on bioflocs.

In contrast, the observations at the Gdynia plant are ambiguous and could not confirm the hypothesis of anoxic DON production from CON hydrolysis. During all the batch experiments, only the DON behaviour in reactor 2 revealed the same trend (i.e. production of $0.04\text{--}0.22 \text{ g N/m}^3$). In the anoxic compartment of the full-scale bioreactor, the behaviour of CON was inconsistent. The concentrations of “high” ($0.45\text{--}1.2 \mu\text{m}$) colloidal subfraction decreased by 0.6 g N/m^3 ($0.45\text{--}1.2 \mu\text{m}$), whereas simultaneously the concentrations of “low” ($0.1\text{--}0.45 \mu\text{m}$) colloidal subfraction increased by 1.1 g N/m^3 (Czerwionka *et al.* 2008).

CONCLUSIONS

Based on the results obtained at the two studied plants, the following conclusions can be derived:

1. The chemical pretreatment of settled wastewater with ZnSO_4 did not adversely affect the observed nitrification rates in the (last) aerobic phase. It caused, however, a reduction of denitrification rates in the anoxic phase. Moreover, the chemical pretreatment did not appear to generally decrease DON but decreased CON.

2. The inorganic N mass balances revealed N losses up to approximately 10% which could be attributed to a few novel pathways of nitrogen removal, most likely aerobic denitrification or simultaneous nitrification/denitrification.
3. DON was explicitly produced in the aerobic phase during all the experiments.
4. Organic nitrogen conversion occurred at a significant rate in the anoxic phase with biodegradable COD consumption and solids hydrolysis.

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