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# The treatment of anaerobic digester supernatant by combined partial ammonium oxidation and denitrification

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## ABSTRACT

Although the most common method of municipal reject water management is treating in the main flow of a wastewater treatment plant, an alternative method is purifying it separately in a side-stream. This helps to reduce the load of nitrogen and carbon entering the sewage treatment system. This paper reports the results of the application of the SBR for the treatment of anaerobic sludge digester supernatant. At a reduced dissolved oxygen (DO) concentration of 0.7 mg $O_2$/l in the aeration phase, application of mixing phases and pH regulation at the beginning of the SBR cycle (pH 8) allowed to obtain a suitable Anammox influent (50/50 ammonium:nitrite). Applying low oxygen concentrations and a one-hour long mixing phase enabled heterotrophic denitrification in the reactor. The partial use of organic compounds in this process resulted in a COD/N ratio in the effluent of ca. 1, which is favourable for the Anammox influent.

*Keywords:* Anaerobic sludge digester supernatant; SBR; Partial nitrification; Denitrification; Anammox

## 1. Introduction

The supernatant from the anaerobic digestion of sludge (the so-called "reject water") is a good example of highly concentrated ammonium streams (800–1000 mg N–$NH_4$/l) with unfavourable C/N ratio for nitrogen removal by denitrification. This supernatant is usually recirculated to the biological section of an activated sludge plant without pretreatment contributing to 15–20% of the influent nitrogen load [1]. Therefore, it is proposed to purify the supernatant separately in a side stream of the plant rather than return it to the system influent for treatment as a part of the main flow. In the side stream operational parameters can be adjusted that allow nitrogen removal by partial nitrification/denitrification or use in an integrated Sharon/Anammox system. The main product of partial nitrification is nitrite. Because partial nitrification is most often applied for the treatment of wastewater with an adverse C/N ratio, nitrite removal from wastewater requires the introduction of an additional carbon source that increases the costs of the treatment. Partial nitrification of ammonium to nitrite is presented as a possible way to achieve an Anammox influent of the required composition [2]. Anammox (anaerobic ammonium oxidation) is an autotrophic process of nitrogen removal in which ammonium is converted, under anaerobic conditions, directly into nitrogen gas with nitrite as an electron

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acceptor and in the absence of organic carbon sources. Ammonium and nitrite are substrates for Anammox bacteria. The main product of ammonium oxidation under anaerobic conditions is nitrogen gas. However, about 10% of the input substrate is converted to nitrate nitrogen. Partial nitrification using a SHARON reactor combined with Anammox process has been successfully studied in recent years [3–5].

The Sharon process generates effluent with the ammonium to nitrite ratio of 1 that is suitable to serve as an influent to an Anammox reactor [6]. Waki et al., treating swine wastewater, showed that the pH of the sludge was the most important factor affecting Anammox performance [7]. A pH in the range of 6.6–8.1 retained Anammox activity, whereas lower or higher did not. The Anammox process requires no additional carbon source, because it is driven by autotrophic bacteria. Yang et al. proved however the positive impact of inorganic carbon on Anammox activity [8]. Moreover, obtaining the correct composition of sewage for input the Anammox process requires the Sharon process to be conducted at a high temperature. The usefulness of the SBR for partial nitrification in the reject water was reported by [9]. According to these authors, both SBR and chemostat could be effectively used to produce a mixture suitable for an Anammox process. However, in case of SBR, proper effluent composition was obtained at DO concentration >3 mg $O_2$/l, sludge retention time (SRT) of 5 d and at the temperature of 30°C. Similarly, Zeng et al. obtained partial nitrification at 30°C in a two-stage sequencing batch reactor system [10]. However, carrying out the process in such temperature requires reactor heating that entailed high operating costs. Therefore, in the present research we propose a combination of partial nitrification at low DO concentration (up to 0.7 mg $O_2$/l), pH ca. 8 and ambient temperature, and heterotrophic denitrification. Partial nitrification at ambient temperature was obtained by other authors however the process was carried out in continuous-flow reactor with the attached biomass or in granular sludge systems [11–13].

In our study, mixing phases were applied to achieve denitrification that uses carbon compounds and consequently C/N is descended. Such operating conditions lead to the outflow with the appropriate C/N ratio and ammonia nitrogen to nitrite nitrogen ratio suitable for the Anammox process.

Nitrite accumulation can be achieved by maintaining the DO at low concentration since the Monod oxygen saturation coefficients of the kinetics for nitritation and nitratation are known to be 0.3 and 1.1 mg/l, respectively [14]. Reject waters are characterized by a low alkalinity/ammonium ratio which would provide a partial nitrification [15]. The key parameter to obtain partial nitrification is the bicarbonate/ammonium ratio. The molar stoichiometric ratio for complete nitrification is 2 mol $HCO_3^-$/mol $NH_4^+$. This ratio in reject water is about 1, which results in a natural pH decrease when approximately 50% of ammonium is oxidized. However, in our research the reaction was maintained at 8 pH at the beginning of the reactor cycle in an attempt to prevent inhibition of partial nitrification at the level below 50% by excessive lowering of pH. In addition, a high pH favours the formation of free ammonia (FA). A high concentration of FA effectively inhibits nitrite oxidation, resulting in an accumulation of nitrite.

In the present research, mixing phases were introduced to the SBR cycle. The low DO concentration, one-hour mixing phases and the presence of easily degradable organic carbon in wastewater should foster heterotrophic denitrification, even more so since the literature data indicate that the reduction of nitrites requires less organic carbon compounds than nitrate reduction in classic denitrification.

The main goal of this research was to determine whether the application of low DO concentration and pH regulation in a SBR during the real reject water treatment would allow a properly constituted Anammox influent to be obtained. In addition, it was checked whether the low concentration of oxygen and alternating aerobic and anaerobic phases in the SBR favoured denitrification. Moreover, we examined if, under these conditions, biodegradable organic compounds involved in reject water were used by activated sludge for nitrate reduction.

## 2. Material and methods

### 2.1. Process configuration

The experiment was carried out in three SBRs, each with a working volume of 5 l, operated in parallel. Before each series, the SBRs were seeded with sludge from a municipal wastewater treatment plant with simultaneous nitrification and denitrification. The reactors were equipped with a stirrer with the regulated rotation speed (50 rpm) and a controlled air supply system. Air was supplied by porous diffusers placed at the bottom of the tank. The amount of air entering the sequencing batch reactors was automatically adjusted to a stable set-point of 0.7 mg $O_2$/l. The pH was maintained at the level of about 8 at the beginning of the reactor cycle. The reactors were operated at a temperature of 20°C. Total suspended solid concentration varied from 4320 to 5720 mg TSS/l, with VSS accounting for 65%.

### *2.2. Experiment organization*

During the experiment three series differing in volumetric exchange rate (*n*) were conducted (0.1 $d^{-1}$, 0.3 $d^{-1}$ and 0.5 $d^{-1}$). In each series anaerobic sludge digester supernatant was introduced to the reactors. The SBRs were operated in a 24 h cycle mode. Each cycle consisted of 8 following phases: filling, I aeration, I mixing, II aeration, II mixing, III aeration, settling and decantation. The lengths of the filling phase (0.25 h), settling (0.5 h) and decantation (0.25 h) were stable for each series. The lengths of each aeration and mixing phase were 7 h and 1 h, respectively.

### *2.3. Characteristic of anaerobic sludge digester supernatant*

Anaerobic sludge digester supernatant was obtained from open fermentation basins of the Municipal Wastewater Treatment Plant in Olsztyn. Average values of pollutant concentrations in anaerobic sludge digester supernatant were: 1611.4 ± 108 mg COD/L, 728.9 ± 49.8 mg $N–NH_4$/L, 939.4 ± 11.4 mg TKN/L. Organic nitrogen comprised 22% of the total Kjeldahl nitrogen (TKN). The value of the COD/N ratio of anaerobic sludge digester supernatant was about 1.7.

### *2.4. Analytical methods*

In each series the adaptation period lasted about 30 d and was considered complete when the range of changes of particular parameters in the effluent (COD, TKN, $N–NH_4$, $N–NO_2$, $N–NO_3$) within 7 d did not exceed 5–10%. At established effluent parameters, the research was carried out to determine the COD removal rate, ammonia removal rate and nitrification rate. In the working cycle of the reactor, periodic sampling and measurements of COD and nitrogen compounds were performed.

Concentration of free ammonia (FA) (mg N/l) and free nitrous acid (FNA) (mg N/l) were calculated as a function of pH, temperature and total ammonium nitrogen (TAN), for FA, or total nitrite ($TNO_2$), for FNA [16 after 17]:

$$\mathrm{FA} = \frac{\mathrm{TAN}}{1 + (10^{-\mathrm{pH}}/K_e^{\mathrm{NH}})} \quad \text{where} \quad K_e^{\mathrm{NH}} = e^{-6344/(273+T)} \tag{1}$$

$$\mathrm{FNA} = \frac{\mathrm{TNO_2}}{1 + (K_e^{\mathrm{NO}}/10^{-\mathrm{pH}})} \quad \text{where} \quad K_e^{\mathrm{NO}} = e^{-2300/(273+T)} \tag{2}$$

### *2.5. Chemical analyses*

Daily measurements of pollutant concentration in the effluent from the reactors included: COD, TKN, ammonia nitrogen, nitrites and nitrates. The activated sludge was analysed for total suspended solids (TSS), volatile suspended solids (VSS) and sludge volume index (SVI). The analyses were performed according to APHA [18].

## 3. Results and discussion

Changes in the ammonium concentration and forms of oxidized nitrogen ($NO_x$) in the SBR cycle in series 1–3 are shown in Fig. 1. Ammonium removal in the SBR cycle proceeds according to zeroth-order kinetics that means linear changes of ammonium concentration in time.

In parallel with increasing volumetric exchange rate (*n*), the values of the rate constants of ammonium

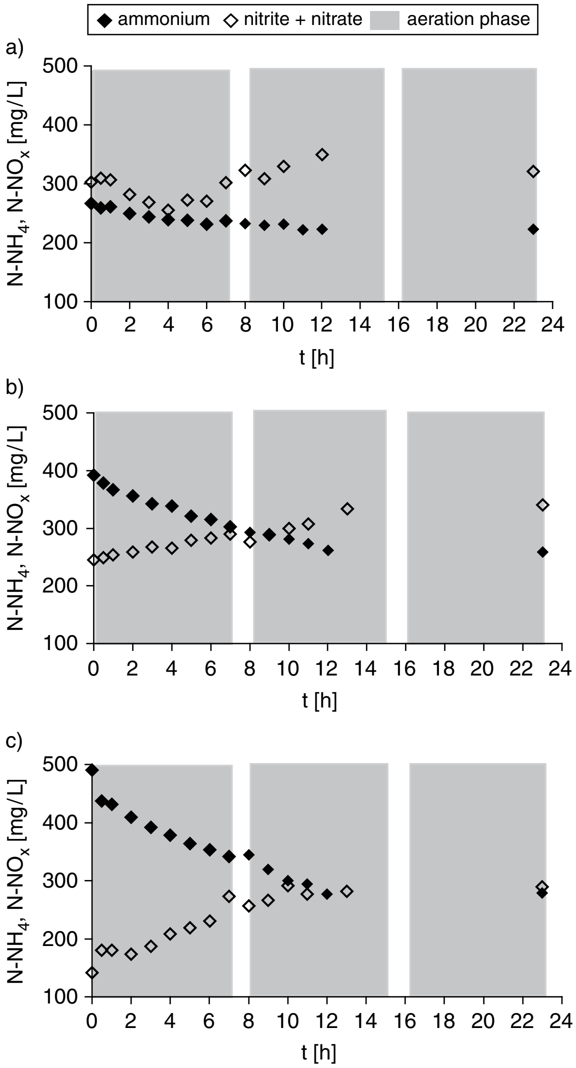

Fig. 1. Ammonium removal and nitrite and nitrate formation in the SBR cycle; a) series 1, b) series 2, c) series 3.

removal increased; however, their values in the I aeration phase were higher than those obtained in the II aeration phase. Ammonium nitrogen concentrations in the effluent were 223.1 mg N–$NH_4$/l, 259 mg N–$NH_4$/l and 279 mg N–$NH_4$/l at *n* of 0.1 $d^{1}$ (series 1), 0.3 $d^{-1}$ (series 2) and 0.5 $d^{-1}$ (series 3), respectively.

The rate of ammonia removal in the I phase of aeration varied from 1.46 mg N–$NH_4$/g VSS·h at the volumetric exchange rate of 0.1 $d^{-1}$ (series 1) to 5.58 mg N–$NH_4$/g VSS·h at the volumetric exchange rate of 0.5 $d^{-1}$ (series 3) (Table 1).

Independent of the volumetric exchange rate, ammonium nitrogen was oxidized to nitrite indicating that the II phase of nitrification was inhibited. In the effluent the highest nitrate concentration (5.5 mg N–$NO_3$/l) was observed in series 1 at the volumetric exchange rate of 0.1 $d^{-1}$. In the remaining series nitrate concentrations in the treated waters did not exceed 2 mg N–$NO_3$/l, while the nitrite concentration was ca. 300 mg N–$NO_2$/l.

From Fig. 1 it can be seen that during the first 6 h of aeration ammonium removal was not accompanied by a simultaneous stoichiometric increase in the nitrite and nitrate concentration. A part of the ammonium nitrogen was probably used for a biomass synthesis. However, taking into consideration the parallel decrease in the concentration of organic compounds (Fig. 2) and the low DO concentration in the aeration phase it can be assumed that the loss of nitrogen also resulted from heterotrophic denitrification. This is supported by the fact that all the organic compounds removed in the cycle were eliminated at that time. It is known that anaerobic sludge digester supernatant contains a high concentration of organic compounds. In the supernatant used in the experiment only part of organic compounds was biodegradable (COD – 1611 mg/l, BOD – 692 mg/l). On the other hand, organic compounds expressed as BOD were easily accessible to microorganisms as confirmed by the high value of the rate constant of biochemical oxygen demand removal ($k = 0.6\ d^{-1}$).

The $BOD_5$/COD ratio in the reject water was ca. 0.43 meaning that the organics were recalcitrant. Therefore, irrespective of the volumetric exchange rate, the effectiveness of COD removal was low (about 54–59%) corresponding to the high COD concentration in the effluent of 728 mg COD/l ($n = 0.1\ d^{-1}$) and about 660 mg COD/l ($n = 0.3, 0.5\ d^{-1}$). An additional factor influencing organics removal was the low DO concentration (below 0.7 mg $O_2$/l).

Investigation of COD concentration changes in the SBR cycle showed that this process proceeds according to first-order kinetics.

Table 1
The rate of ammonium nitrogen removal in series 1–3

| Series | Reaction rate (mg/l h) I aeration | Reaction rate (mg/l h) II aeration | Reaction rate (mg/g VSS h) I aeration | Reaction rate (mg/g VSS h) II aeration |
|---|---|---|---|---|
| Series 1 | 4.08 | 2.56 | 1.46 | 0.91 |
| Series 2 | 11.97 | 7.60 | 4.36 | 2.75 |
| Series 3 | 20.60 | 15.96 | 5.58 | 4.33 |

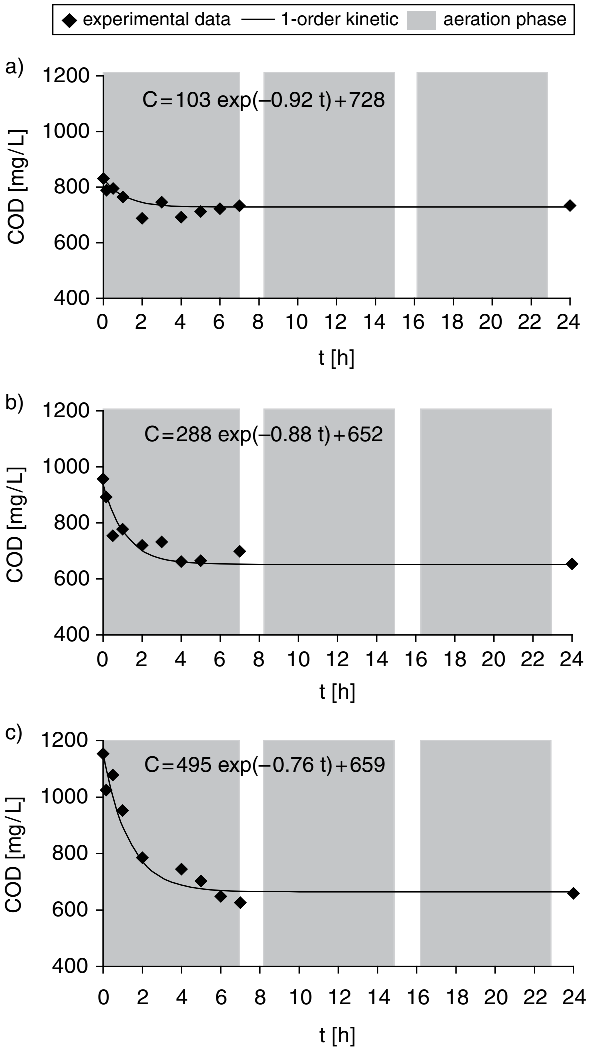

Fig. 2. COD removal during the SBR cycle; a) series 1, b) series 2, c) series 3.

Rapid loss of COD was observed during the first two-three hours of the cycle, then the COD concentration remained at a nearly constant level (Fig. 2). The rate constants of COD removal were in the range of 0.92–0.76 $h^{-1}$.The lowest initial COD removal rate, ca. 33.8 mg/g VSS·h was observed at $n = 0.1\ d^{-1}$, the highest – 101.9 mg/g VSS ·h at $n = 0.5\ d^{-1}$.

Table 2
Parameters in the effluent

| $n$ (d$^{-1}$) | Effluent | | | | | COD/TN | N–NH$_4$: N–NO$_2$ |
|---|---|---|---|---|---|---|---|
| | N–NH$_4$ (mg/l) | N$_{org}$ (mg/l) | N–NO$_2$ (mg/l) | N–NO$_3$ (mg/l) | COD (mg/l) | | |
| 0.1 | 223.1 ± 41.1 | 81.2 ± 20.9 | 321.0 ± 41.6 | 5.5 ± 1.9 | 734 ± 78.8 | 1.16 | 1:1.44 |
| 0.3 | 259.0 ± 20.8 | 52.0 ± 21.0 | 340.5 ± 28.6 | 2.0 ± 1.3 | 654 ± 91.1 | 1.00 | 1:1.31 |
| 0.5 | 279.0 ± 44.2 | 49.0 ± 22.8 | 289.2 ± 32.6 | 2.1 ± 0.7 | 659 ± 50.3 | 1.06 | 1:1.04 |

Table 2 summarizes the effluent parameters after the treatment of anaerobic sludge digester supernatant in an SBR. At the limited oxygen concentration in the aeration phases (0.7 mg O$_2$/l) and at the ammonium nitrogen concentration in the influent of ca. 730 mg N–NH$_4$/l limitation of ammonium oxidation occurred. The nitrification efficiency was 16% at the volumetric exchange rate of 0.1 d$^{-1}$, while with increasing $n$ the nitrification efficiency increased to about 43% at $n$ of 0.5 d$^{-1}$ (Fig. 3). Merely partial nitrification to nitrite was achieved that may result from an inhibitory environment caused by FA and FNA. The calculated values of FA and FNA showed that only the concentrations of FA were >3.5 mg N–NH$_3$/l - an inhibitory value reported for nitrite oxidizing bacteria (NOB) [17]. In the present study FA concentration was on the level of 14.3, 21.1 and 26.4 mg N–NH$_3$/L in series 1, 2, 3, respectively. Concerning FNA, calculated concentrations were below 0.01 mg N–HNO$_2$/l, and confronted with the values of 0.06–0.85 mg N–HNO$_2$/l, proposed by Anthonisen et al., cannot be regarded as inhibitory concentrations [17]. Thus, the low formation of nitrate in the effluent might be explained by the FA inhibition of NOB. Anthonisen et al. also reported an inhibitory effect of FA in connection with ammonia-oxidizing bacteria (AOB) that takes place in the FA range of 8.23–123.53 mg N–NH$_3$/l [17]. The percentage of AOB in total bacteria in activated sludge was evaluated using fluorescence in situ hybridization (FISH) and it was shown that AOB fraction in biomass decreased from 0.24% to 0.08% with the increasing volumetric exchange rate (Fig. 3).

A possible explanation for the declining AOB percentage in activated sludge is the FA values that suggest a negative influence on the ammonia-oxidizing bacteria community. A question arises as to why the nitrification efficiency increased from 16 to 43% despite the decrease in the AOB fraction of the biomass. It can be assumed that with increasing $n$ autotrophic nitrification was replaced by heterotrophic nitrification. The higher the value of $n$ the more organic compounds were introduced to the reactor. Compared to autotrophic nitrifiers, heterotrophic nitrifiers require lower DO concentration, tolerate more acidic environment and prefer higher C/N ratios [19]. It was proved that media supporting the growth of heterotrophic nitrifiers must contain organic carbon in addition to nitrogen [20]. According to Patureau et al., despite the higher affinity of autotrophs for ammonium compared to heterotrophs, the autotrophic nitrifying activity decreased in the presence of organic carbon sources, because of a higher heterotrophic cell number and competition for oxygen and ammonium [21].

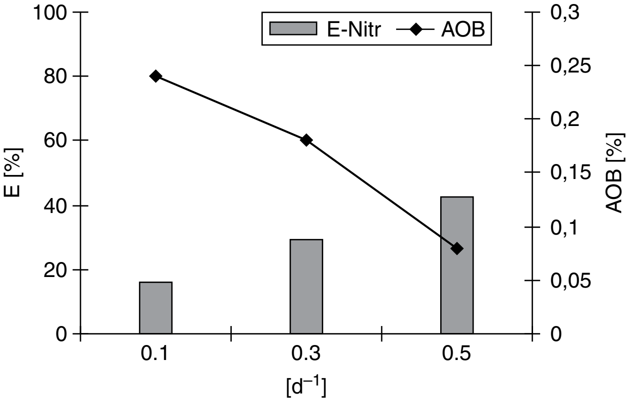

Fig. 3. AOB percentage in activated sludge in relation to volumetric exchange rate.

As mentioned above, nitrogen removal from the wastewater was observed. The amount of nitrogen used by activated sludge in the synthesis of biomass and denitrification was determined. It was assumed that all nitrogen introduced to the SBR is oxidized and used for biomass production and that nitrite and nitrate nitrogen obtained in nitrification in a given SBR cycle and remaining in the reactor after the previous cycle can be denitrified. With increasing volumetric exchange rate the efficiency of nitrogen removal from wastewater increased (Fig. 4). Similarly, Xu et al. obtained significant heterotrophic denitrification (0.11 kg NO$_3$–N/kg·d) during urban landfill leachate treatment in SBR with integrated partial nitrification and Anammox [22].

The efficiency of denitrification at the volumetric exchange rate of 0.1 d$^{-1}$ was about 8%, while the amount of nitrogen removed from wastewater in this process was 27.4 mg N/l. With the increase in $n$, the efficiency of denitrification increased to reach 32.6% at the volumetric

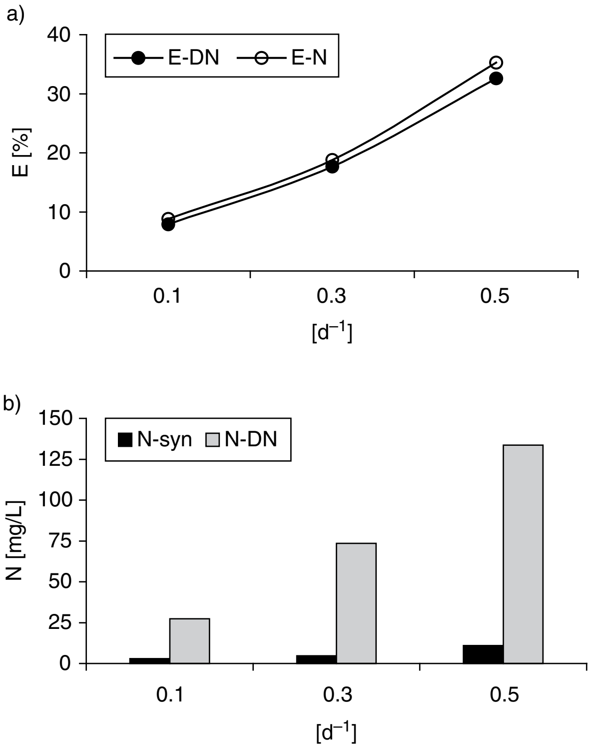

Fig. 4. Efficiency of denitrification (E-DN) and nitrogen removal (E-N) (a), the amount of nitrogen removed by denitrification (N-DN) and for biomass synthesis (N-syn) (b) at different volumetric exchange rate.

exchange rate of 0.5 d$^{-1}$, which corresponded to the highest amount of nitrogen removed in this process –133.7 mg N/l. In the present research, the main nitrification products were nitrites. The ratio of organic compounds to oxidized nitrogen formed at the beginning of the SBR cycle (COD/N–NO$_x$) was calculated. Data in Table 3 show that,with increasing volumetric exchange rate, the COD/N–NO$_x$ ratio increased from 3.01 to 8.1, meaning the increase in the amount of available COD per each gram of nitrite that favoured denitrification.

Heterotrophic denitrification effectively occurs at a low concentration of oxygen and in the presence of organic compounds. Henze showed that, to obtain complete denitrification, the COD/N ratio in wastewater needs to be sufficiently high [23]. For example, 4.2 g COD/g N with glucose as a carbon source is required for nitrogen reduction. Theoretically, it has been shown that, under anoxic conditions and with a biodegradable organic substrate present in the wastewater, COD consumption is 2.86 mg to reduce 1 mg of N-nitrate. Since a combined nitrification/denitrification proceeded, COD/N requirements in practice are higher (in the range of 5–10 g COD/g N) and a minimum ratio of 3.5–4 g COD/g N appears necessary [23]. Kuba et al. indicated that, with an influent COD/N ratio <3.4 g COD/g N, an extra COD should be added to remove residual nitrate [24]. Denitrification of nitrites requires a lower amount of organic compounds for the reduction of each gram of nitrites in comparison with denitrification proceeding from nitrates. In our research it was shown that independently of the volumetric exchange rate for the reduction of 1 g of N–NO$_x$ an average amount of 3.73 g COD (2.68 g BOD) is required.

Table 3
The ratios of organics/N–NO$_x$ achieved in the presented study

| *n* (d$^{-1}$) | COD/ N–NO$_x$* | COD/ N–NO$_x$** | BOD/ N–NO$_x$** |
|---|---|---|---|
| 0.1 | 3.01 | 3.54 | 2.55 |
| 0.3 | 3.93 | 4.05 | 2.86 |
| 0.5 | 8.10 | 3.59 | 2.62 |
| Mean value | – | 3.73 | 2.68 |

*The values in the reactor at the beginning of the SBR cycle.
**The amount of grams of COD or BOD used per gram of N–NO$_x$ reduced during the SBR cycle.

The idea of coupling the partial nitrification with Anammox process has been suggested as one of the most economical processes [3,25]. However, Anammox is not suitable for wastewaters with ratios of C/N > 1 since under these conditions Anammox bacteria are no longer able to outcompete heterotrophic denitrifying bacteria [26]. The anaerobic sludge digester supernatant used in the study was characterized by a C/N ratio of about 1.7 and the high ammonium concentration.

Application of limited oxygen concentration in the aeration phase (DO < 0.7 mg O$_2$/l) in the SBR cycle favoured denitrification, that contributed to the use of organic compounds. As a result, the ratio of COD/N obtained in the effluent was ca. 1.

## 4. Conclusions

Treating reject water in the SBR at the low DO concentration in the aeration phase, results in great savings in aeration costs, and can be effectively used to produce a 50/50 ammonium-nitrite mixture suitable for a subsequent Anammox process. Our results demonstrated the feasibility of the present technology as a previous step in the Anammox process. An additional advantage of conducting the process at low oxygen concentration with a one-hour mixing phase is the occurrence of heterotrophic denitrification – for *n* equal to 0.5 d$^{-1}$, its effectiveness reached 32%.

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