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Purification of nitrogen-rich digester supernatant by activated sludge from a membrane bioreactor

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ABSTRACT

The potential of activated sludge from a membrane bioreactor (MBR) to remove nitrogen from anaerobic digester supernatant was tested at various volumetric exchange rates (n) (0.1, 0.2 and 0.3 d^{-1}) and dissolved oxygen concentrations (DO) (0.5, 2.5 and 3.0 mg/L). The high abundance of ammonium-oxidizing bacteria (7.2%) in the sludge caused the ammonium removal efficiency to exceed 97%, independently of DO and n. However, DO and n affected the process rate; thus, the time necessary for ammonium removal ranged from 4 to 8 h. At DOs of 2.5 and 3.0 mg/L, nitrogen removal reached 79.8%–89.0% although external carbon was not added. The nitrogen removal efficiency was highest (91.5%) at an n of 0.3 d^{-1} and a DO of 0.5 mg/L. Thus, this variant, which had the highest availability of substrate and the lowest availability of oxygen, stimulated the cooperation of aerobic nitrifiers, denitrifiers and anammox microorganisms, which were present in the biomass. The high rate of endogenous oxygen uptake indicated the degradation of intracellularly stored organics, which may have supported denitrification. These results indicate that the biological treatment of nitrogen-rich supernatant by highly concentrated MBR sludge in a side-stream is an advantageous alternative to treatment in the main-stream of wastewater treatment plants.

Keywords: Reject water; Membrane bioreactor; Anammox; Simultaneous nitrification and denitrification

1. Introduction

Anaerobic digester supernatant that comes from sludge dewatering is one of a range of difficult-to-treat reject waters with high nitrogen and low organics concentrations. It is difficult to remove nitrogen from this supernatant by conventional sequential autotrophic nitrification and heterotrophic denitrification in the mainstream of wastewater treatment plant (WWTP) because of its low C/N ratio and the high nitrogen load coming into the mainstream. Therefore, some alternatives were proposed. One of them is shortcut nitrification–denitrification via nitrite, which uses about 25% less oxygen for nitrification and 40% less organics for denitrification, and results in lower sludge production [1]. A solution with a higher rate of nitrogen removal and smaller sludge production is a combination of partial nitrification and anoxic/anaerobic ammonium oxidation (anammox). If partial nitrification/anammox is used in the mainstream of the technological line of municipal treatment, organics can limit the activity of anammox; therefore, organic matter can be concentrated and further valorized into biogas, and then the supernatant can be treated autotrophically [2]. Another promising alternative is the co-operation of aerobic and anaerobic ammonium-oxidizing bacteria and heterotrophic denitrifying bacteria [3]. This alternative is

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most attractive when the treatment is performed in a single reactor in which partial nitrification followed by anammox can support heterotrophic denitrification for the removal of high nitrogen loads without external carbon sources. The advantages of this solution over conventional technologies for nitrogen removal include lower energy consumption, a reduced need for additional carbon sources, minimal sludge production and lower emissions of the greenhouse gases NO and N_2O [4].

The cooperation of aerobic ammonium-oxidizing bacteria (AOB), anammox bacteria and denitrifiers is possible in membrane bioreactors (MBRs), which combine activated sludge treatment and membrane filtration for biomass retention, making this alternative a space-saving solution. In MBRs, the high concentration of biomass results in high volumetric reaction rates and creates anaerobic and anoxic zones for the anammox process and for denitrification with the use of endogenous carbon sources [5]. MBR is a technology that can cope with problems associated with the treatment of reject waters, which are high concentrations of total suspended solids and substantial variations in pollutant concentrations. The conditions in an MBR favor the growth of slow-growing microorganisms such as AOB and anammox bacteria. This has been confirmed by the finding that low organic loadings, large accumulations of active biomass and long solids retention times (over 10 d), all of which are typical in MBRs, create microbial communities with slow-growing bacteria that can remove organic micropollutants better than the communities in conventional systems [6].

The number of WWTPs with MBR technology is increasing [7], and even more, the widespread use of this technology is planned because incorporation of membranes in wastewater treatment offers greater opportunities for water reuse. Although conventional activated sludge systems are still cheaper to use than MBRs, the costs of membranes and MBR operation are decreasing. This means that not only is MBR technology considered one of the best available technologies, but it is also one of the most rapidly developing options in wastewater treatment [8]. In addition to being used in the mainstream of treatment, MBRs can be employed for end-of-pipe polishing of the effluent from existing WWTPs [9] or in the side-stream of treatment [10]. The benefit of the latter two solutions is that the use of the MBR does not interfere with the overall treatment processes in the main-stream of the WWTP.

Despite intensive development of MBRs, this technology has been used mainly for the treatment of municipal wastewater with low concentrations of nitrogen (40 mg TN/L) (TN – total nitrogen), in which the influent was split into an aerobic tank and an anoxic tank [11], and in small WWTPs, in which anaerobic stabilization of sludge is not profitable. Now, MBRs are also operated in large facilities [12] where problems with anaerobic supernatant are important. However, the use of MBRs for nitrogen removal from digester supernatant, particularly in side streams, has not been widely documented in the literature. Available studies have focused mostly on the treatment of synthetic wastewater or the use of pure carbon sources, such as methanol or acetic acids, for effective denitrification [13]. There is a lack of data about the possibility of using the endogenous activity of highly concentrated activated sludge and the autotrophic activity of microorganisms in the MBR for effective nitrogen removal from the supernatant.

Therefore, the objective of this study was to determine how effectively MBR activated sludge removes nitrogen when it is exposed to digester supernatant, which is rich in nitrogen. The experiments investigated the effect of the volumetric exchange rate (n) and the initial concentration of dissolved oxygen (DO) on the kinetics and efficiency of nitrogen removal from real supernatant without external organics addition by activated sludge from a full-scale MBR treating municipal wastewater. For insight into how the dominant mechanisms of nitrogen removal by MBR activated sludge depends on operational parameters, respirometric and molecular analyses of the activated sludge was also conducted. The results show that MBR activated sludge can be successfully used for the side-stream treatment of nitrogen-rich supernatant.

2. Materials and methods

2.1. Wastewater and activated sludge

The anaerobic digester supernatant was collected from a full-scale municipal WWTP that has a population equivalent of about 342,000. In this WWTP, the supernatant is released from the anaerobically treated mixture of primary and secondary sludge during its dewatering on a press belt after conditioning with a polyelectrolyte. In this supernatant, the average concentrations of pollutants were as follows: 338 ± 48 mg COD/L (COD – chemical oxygen demand), 68 ± 12 mg BOD_5/L (BOD – biochemical oxygen demand), 594 ± 85 mg TN/L, 532 ± 58 mg NH_4–N/L, 119 ± 21 mg TP/L (total phosphorus), 7.5 ± 0.1 pH, and alkalinity of 1,550 ± 125 mg $CaCO_3$/L. The influent COD/N ratio was 0.6.

The activated sludge was collected from a full-scale municipal WWTP that has a population equivalent of about 3,300 and is designed as a plug-flow system with MBR technology. The technological system consists of a mechanical component (5.0 mm screen, a trap for sand and fat, 1.0 mm screen), a biological component (pre-anoxic tank with a hydraulic retention time (HRT) of 18 h; aerobic tank with an HRT of 44 h; post-anoxic tank with an HRT of 20 h) and a membrane unit (capillary ultrafiltration membranes operated in a vacuum system). The sludge age is maintained at about 50 d. In this WWTP, the average annual influent concentrations are 1,133 ± 242 mg COD/L, 661 ± 150 mg BOD_5/L, 129 ± 18 mg TN/L and 16 ± 7 mg TP/L. The average annual effluent (permeate) concentrations are 28 ± 6 mg COD/L, 2 ± 1 mg BOD_5/L, 3 ± 1 mg TN/L and 6 ± 1 mg TP/L. Due to the replacement of a secondary clarifier with a membrane unit, the effluent concentrations of total suspended solids are below 0.2 mg/L.

2.2. Organization of the experiment

Activated sludge taken from the aerobic tank of the MBR system was transported on ice for testing its use in the treatment of anaerobic digester supernatant. For these tests, 2 L of activated sludge was placed in the reactors, which were equipped with stirring and

fine-bubble aeration. The concentration of activated sludge was 19.0 ± 3.7 g MLSS/L (MLSS – mixed liquor suspended solids), of which 76% was organic solids. The experiments were conducted at ambient temperature (approx. 20°C). In stage 1, to determine the effect of the n on the efficiency and kinetics of nitrogen and organics removal, three different doses of the supernatant were added to the reactors, resulting in n values of 0.1 d^{-1} ($R_{0.1\_2.5}$), 0.2 d^{-1} ($R_{0.2\_2.5}$), and 0.3 d^{-1} ($R_{0.3\_2.5}$). The reactors were constantly aerated; "2.5" in the series name indicates that the initial concentration of DO in all reactors in the first 2–3 h of the cycle was about 2.5 mg/L. The resulting organics and nitrogen loadings were as follows: 0.002 g COD/(g MLSS d) and 0.004 g TN/(g MLSS d) in $R_{0.1\_2.5}$, 0.004 g COD/(g MLSS d) and 0.008 g TN/(g MLSS d) in $R_{0.2\_2.5}$, 0.007 g COD/(g MLSS d) and 0.012 g TN/(g MLSS d) in $R_{0.3\_2.5}$.

Based on the results of stage 1, n values of 0.2 and 0.3 d^{-1} were selected for testing the effect of DO concentration on the efficiency and kinetics of nitrogen and organics removal (stage 2). The following series were conducted: n = 0.2 d^{-1} and an initial DO concentration of 3.0 mg DOL ($R_{0.2\_3.0}$); n = 0.2 d^{-1}, 0.5 mg DO/L ($R_{0.2\_0.5}$); n = 0.3 d^{-1}, 3.0 mg DO/L ($R_{0.3\_3.0}$); and n = 0.3 d^{-1}, 0.5 mg DO/L ($R_{0.3\_0.5}$). All experiments were conducted in duplicate.

Due to the fact that the supernatant did not contain the biodegradable organic matter necessary for a high rate of denitrification, each experimental series lasted 24 h to provide sufficient time for denitrification with the use of endogenous organic matter, which is a slower process than that with the use of organics present in wastewater. During the 24 h experiments, samples of activated sludge were taken from the reactors at specified time intervals. These samples were filtrated with a 0.45 μm filter, and the filtrate was analyzed to determine the concentrations of COD, TN, ammonium, nitrite and nitrate, as well as the pH and alkalinity. On the basis of these results, the changes in pollutant concentrations over time were determined, as were the kinetic parameters of pollutant removal, that is, the rate constants and rates of ammonia removal. In addition, the respirometric activity of the sludge from the MBR was measured after its exposition to digester supernatant to determine the specific oxygen uptake rates of exogenous respiration, nitrification and endogenous respiration. Moreover, the composition of activated sludge from the MBR was investigated with the use of fluorescence *in situ* hybridization (FISH) and high-throughput sequencing (NGS).

2.3. Analytical methods and calculations

The concentrations of COD, TN, nitrite and nitrate were measured spectrophotometrically with LCK 614, LCK 338, LCK 342, and LCK 340 tests (Hach Lange GmbH, Germany). Concentrations of ammonium nitrogen were measured with a distillation method [14]. Alkalinity and pH were measured with a TitroLine. The concentrations of MLSS and mixed liquor volatile suspended solids (MLVSS) were determined according to APHA [14]. DO in the activated sludge was measured with a ProODO optical oxygen meter (YSI Environmental).

The respirometric activity of the activated sludge was measured using an OxiTop control respirometric unit (OC 110, WTW), according to Zielińska et al. [15]. The organics and nitrogen loadings in the measuring vessels were the same as those in reactors $R_{0.1\_2.5}$, $R_{0.2\_2.5}$, and $R_{0.3\_2.5}$. This analysis was performed in triplicate.

The nitrite accumulation ratio (NAR) was expressed as a percentage after dividing the nitrite concentration by the sum of the nitrite and nitrate concentrations in the effluent.

To test the differences between series for statistical significance and to calculate the rate constants for oxygen uptake and for removal of COD and nitrogen forms, Statistica 13.1 (Statsoft) was used.

The abundance of bacteria in the MBR activated sludge was investigated with FISH. Immediately after transportation on ice, the activated sludge was fixed and then selected microorganisms were identified with the use of the following molecular probes: Nso190 (ammonium-oxidizing *Betaproteobacteria*), NIT3 (*Nitrobacter* sp.), Ntspa662 (*Nitrospira* sp.) and Amx368 (anammox bacteria). The conditions used for applying these probes are in probeBase (www.microbial-ecology.net/probebase). To mount the samples before visualization with a Nikon Eclipse epifluorescence microscope (Nikon, Japan), VECTASHIELD (Vector Laboratories, Great Britain) was used. The percent abundance of detected bacteria was quantified by image analysis using ImageJ software (http://rsb.info.nih.gov/ij/) and calculated as the bioarea fraction of the targeted microbial population (stained by the specific probe) relative to that of the total microbial community (stained by the universal probe EUBmix), based on examination of 24 fields of view for each probe. For FISH quantification, three replicates per sample were analyzed. The coefficients of variation for all values with each probe were 15%–20%.

From the MBR activated sludge sample, DNA was isolated using a FastDNA® SPIN Kit for Soil (MP Biomedicals, USA). The quality and quantity of the DNA was analyzed with Lite NanoDrop spectrometer (Thermo Scientific, USA). The V4 hypervariable region of the 16S rRNA gene was amplified using the 926wF/1392R (5′-AAACTYAAA KGAATTGRCGG-3′/5′-ACGGGCGGTGTGTRC-3′) universal primer set [16] targeting of the Bacterial, Archaeal and Eukaryotic 16S rDNA gene.

Sequencing was done using the MiSeq Illumina platform as described in Świątczak et al. [17]. The sequences have been deposited in the NCBI Sequence Read Archive (SRA) as the experiment entitled "Nitrogen removal by MBR sludge" (Accession: PRJNA549156).

3. Results and discussion

3.1. Removal of nitrogen

In the digester supernatant, ammonium nitrogen accounted for almost 90% of TN and the rest was organic nitrogen. Alkalinity was about 400–650 mg $CaCO_3$/L after mixing the supernatant with sludge at the beginning of all experimental cycles. Alkalinity was lowest after about 3 h of the reaction (30–65 mg $CaCO_3$/L), but it was never completely utilized. From the 4^{th}–8^{th} hour, alkalinity increased, reaching twice its initial value at the end of the cycle. Thus, there was no need to adjust alkalinity to maintain a high rate of nitrification of nitrogen-rich supernatant by the activated sludge from the MBR.

In all reactors, NH_4^+–N concentrations changed according to 0-order kinetics (Figs. 1 and 2). An increase in biomass growth was not observed because of the very low organic loadings which are typical for MBRs. Therefore, ammonium uptake for biomass growth was not considered, and it was assumed that ammonium removal was due to oxidation only. In stage 1, in $R_{0.2\_2.5}$ and $R_{0.3\_2.5}$, the NH_4^+–N concentrations partially dropped during 6 h of aeration, at rates of 11.8 and 17.0 mg/(L h), finally reaching 4.2 and 12.6 mg/L, respectively, after 24 h of aeration (Fig. 1 and Table 1). In $R_{0.1\_2.5}$, with the lowest nitrogen loading, ammonium was completely removed after 4 h of aeration, at a rate of 14.8 mg/(L h). Finally, after 24 h, the efficiency of NH_4^+–N removal ranged from 97.6% in $R_{0.3\_2.5}$ to 100% in $R_{0.1\_2.5}$. Changes in DO concentration in the activated sludge corresponded to the gradual utilization of oxygen for nitrification (Fig. 1). After initial depletion, the DO concentration started to increase slowly after the NH_4–N concentrations decreased to about 50 mg/L, despite a further decrease of ammonia concentration. Similarly, high nitrification efficiency (96%) in MBR biomass was obtained by Xing et al. [18] but at the higher DO concentrations, typical for MBRs. In MBRs in general, the sufficient high aeration rate in the membrane tanks can lead to oxygen concentrations of 6 mg/L in the return flow [19]. High efficiencies were also observed in MBRs treating landfill leachate, which is similar to digester supernatant in terms of its high nitrogen concentration and low COD/N ratio, but after long sludge adaptation. For example, Mahmoudkhani et al. [20] reported an average ammonium removal efficiency of 99% at a DO concentration of 3.2 mg/L after a minimum of 55 d of adaptation, and Ghyoot et al. [13] reported an efficiency of 90% at a DO concentration of 1.5 mg/L after about 16 d of adaptation. It was additionally emphasized that ammonium removal was higher in the MBR sludge than in conventional activated sludge, but only during transient shock loads [21]. Taking into account the fact that the activated sludge from the MBR in the present study was not adapted to ammonium-rich digester supernatant, it can be concluded that, in all tested variants, the nitrification efficiency was high at an initial DO concentration of 2.5 mg/L. Therefore, the present study indicates that MBRs can be advantageous solutions for the treatment of ammonium-rich anaerobic supernatant by not adapted biomass because the risk of process failure during low temperature or low DO is minimalized as a result of effective retention of slowly growing nitrifiers.

In stage 2, the initial DO concentration in the reactors did not influence the efficiency of ammonium removal (about 99% after 24 h in all reactors, Table 1); however, it affected the rate of the process, and in consequence, the time required for full oxidation (Fig. 2). The effect of DO was more visible at n of 0.3 d^{-1} than at n of 0.2 d^{-1}. At 0.3 d^{-1}, during 6 h of reaction at 3.0 mg DO/L, NH_4^+–N concentration decreased at a rate of 10.6 mg/(L h). A DO of 0.5 mg/L inhibited the metabolic activity of ammonium oxidizers, which dramatically decreased the ammonium oxidation rate to 3.3 mg/(L h) but not the ammonium removal efficiency. This is in contrast to the study of Zeng et al. [22], which was conducted at the same DO concentration but with a shorter reaction time, so that the reduction in the rate of ammonium oxidization was accompanied by a decrease in the efficiency of ammonium removal. In the present study, at the end of the cycle in $R_{0.3\_3.0}$ and $R_{0.3\_0.5}$, NH_4^+–N concentrations were 5.0 and 3.9 mg/L, respectively. Because the rate of ammonium removal at 3.0 mg DO/L was lower than that at 2.5 mg DO/L in stage 1 at the same n value, in practice, to save energy, an initial DO concentration of 2.5 mg/L should be used. At n of 0.2 d^{-1}, after 6 h of the reaction, NH_4^+–N concentrations decreased at rates of 13.7 and 11.6 mg/(L h) in $R_{0.2\_3.0}$ and $R_{0.2\_0.5}$, respectively. At n of 0.2 d^{-1}, results obtained in both series indicated that the rates of ammonium removal were the same at DO concentrations of 0.5 and 2.5 mg/L, but were higher at 3.0 mg/L. Such results are ambiguous because various factors affect the performance of the MBRs. Due to the smaller size of the flocs in MBRs, substrates are more available than in conventional activated sludge [21]. In addition, nitrifier clusters can grow at different places in the flocs; Munz et al. [23] reported the presence of AOB aggregates on the surfaces of MBR flocs.

In all experimental reactors, except for $R_{0.3\_0.5}$, the changes in ammonium concentrations indicated that even a sudden increase in ammonia load in the influent should not disrupt safe operation when activated sludge is used in MBR technology. Depending on the loading, from 4 h to more than 8 h was necessary to remove ammonium. Therefore, it would be possible to shorten the reaction period by about 50% and still maintain efficient ammonium removal. Despite the fact that the activated sludge used in MBR technology has a long sludge age, the sludge used in this experiment had a high percentage of active biomass, as indicated by the MLVSS/MLSS ratio of 0.76 that ensured efficient ammonium oxidation. The treatment of reject waters with a high ammonium concentration that came from a sludge thickening process, resulted in enrichment of fast-growing AOB, mainly *Nitrosomonas eutropha*, to 10% of all bacteria, thus contributing to the stability of nitritation in main-stream treatment [24]. This enrichment may be even more intensive under MBR conditions, in which slow-growing bacteria are retained in the system. In the present study, the relative abundance of AOB in the activated sludge was 7.2% and AOB predominated among nitrifiers. The number of AOB that were maintained in the system was sufficient for effective nitrification, even without adaptation of activated sludge to high ammonium concentrations.

The changes in nitrite and nitrate concentrations indicated that both AOB and nitrite-oxidizing bacteria (NOB) were present in the MBR sludge. Nitrite concentrations were highest after 5 h of aeration in $R_{0.2\_2.5}$ and $R_{0.3\_2.5}$, and after 3 h of aeration in $R_{0.1\_2.5}$ (Fig. 1). Then, they started to decrease, and no nitrites were detected in the effluents. In all these reactors, nitrate concentrations increased for about 5–6 h and then remained at the same level up to the end of the experiment, despite the simultaneous decrease in nitrite concentration. In $R_{0.3\_3.0}$ and $R_{0.2\_3.0}$, which had higher DO concentrations than the other reactors, the concentrations of nitrites were higher than that of nitrates (Fig. 2). At the lowest DO concentration, a slight accumulation of nitrites and nitrates in the effluent was observed, up to 19 mg/L and 4.7 mg/L in $R_{0.2\_0.5}$, respectively. Low DO limits both AOB and NOB; however, because AOB are better oxygen competitors than NOB, provided that ammonium concentration is sufficient in the environment [25], high efficiency

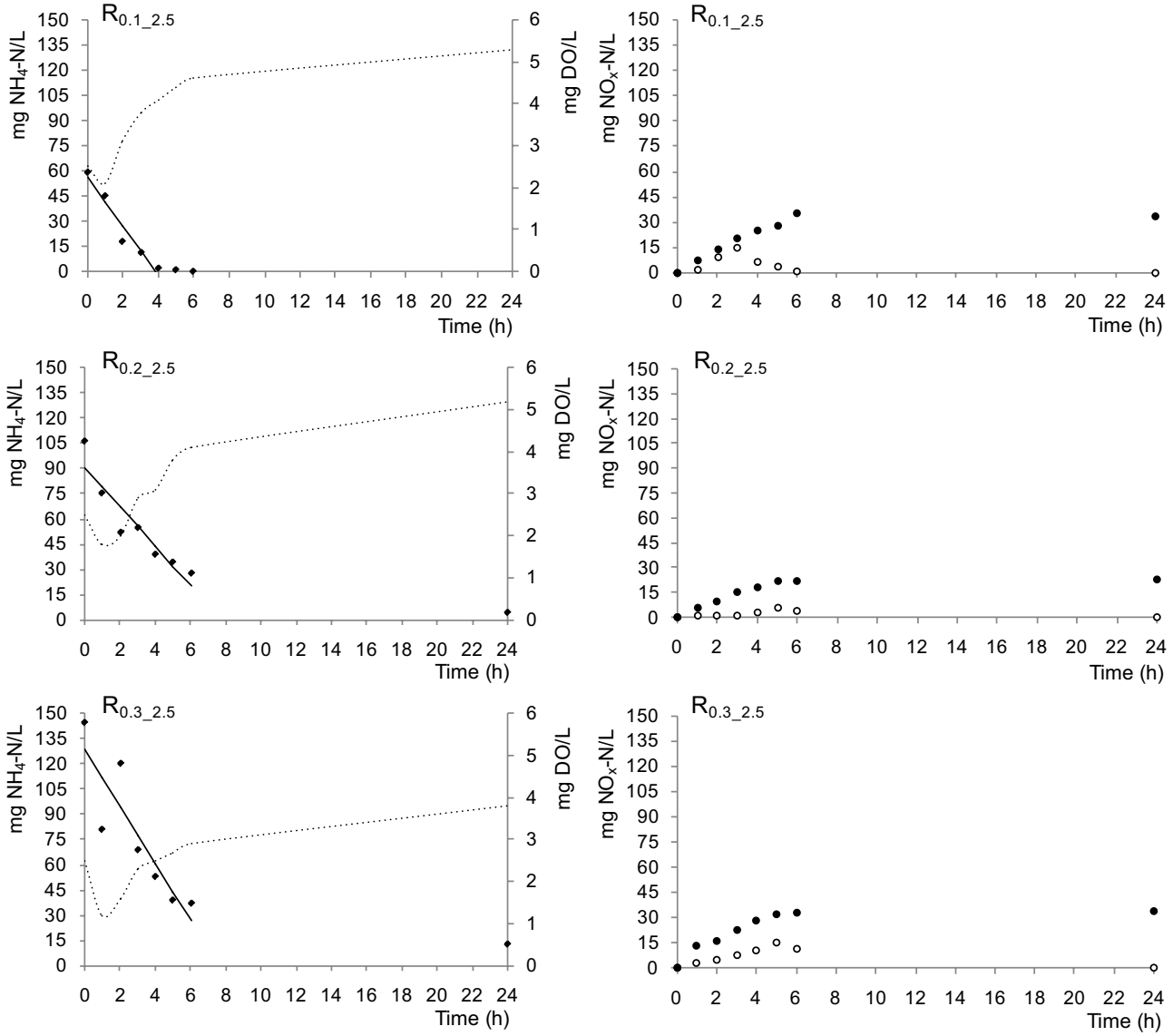

Fig. 1. Changes in ammonium and dissolved oxygen concentrations over time in stage 1 (black diamonds – ammonium concentrations, solid line – 0-order kinetics, dotted line – dissolved oxygen concentrations) and changes in nitrites (white circles) and nitrates (black circles) concentrations over time in $R_{0.1\_2.5}$, $R_{0.2\_2.5}$, and $R_{0.3\_2.5}$.

of ammonium removal was observed. This could have also been determined by the microbial composition of activated sludge. FISH results with regard to the NOB showed that rapid-growing *Nitrobacter* sp. accounted for 2.6% of all bacteria in the MBR activated sludge, and slower-growing *Nitrospira* sp. accounted for 1.0%. Due to effective retention of biomass, MBRs favor the growth of *Nitrobacter* sp. that prefer to exist in the form of suspended cells instead of attaching to activated sludge flocs. Therefore, in conventional WWTPs, *Nitrobacter* sp. are washed out faster than *Nitrospira* sp., which are the dominant NOB and can constitute up to 24% of the biomass [26] because they are better adapted to the low DO and shorter sludge ages [27]. In addition, in an MBR plant from which activated sludge was collected, the sludge was exposed to alternating aerobic (with high DO concentration) and anaerobic conditions. Such conditions tend to select for faster nitrifiers [28]. *Nitrobacter* sp. are so-called "*r*-strategists", which thrive if nitrite and oxygen are present in higher concentrations, grow quickly with high concentrations of substrates and perform reactions at high rates, whereas *Nitrospira* sp. are "*K*-strategists", which are adapted to low nitrite and oxygen concentrations, and grow slowly, but can survive long periods of starvation [29]. Therefore, a low DO of 0.5 mg/L in the present experiments could have inhibited the activity of *Nitrobacter* sp., which resulted in the accumulation of nitrites.

The amount of ammonium removed was not equivalent to that of nitrites and nitrates produced. TN was removed despite constant aeration indicating that simultaneous nitrification and denitrification took place. The efficiencies of TN removal, calculated based on the initial concentrations of TN in the raw digester supernatant, ranged from 74 to

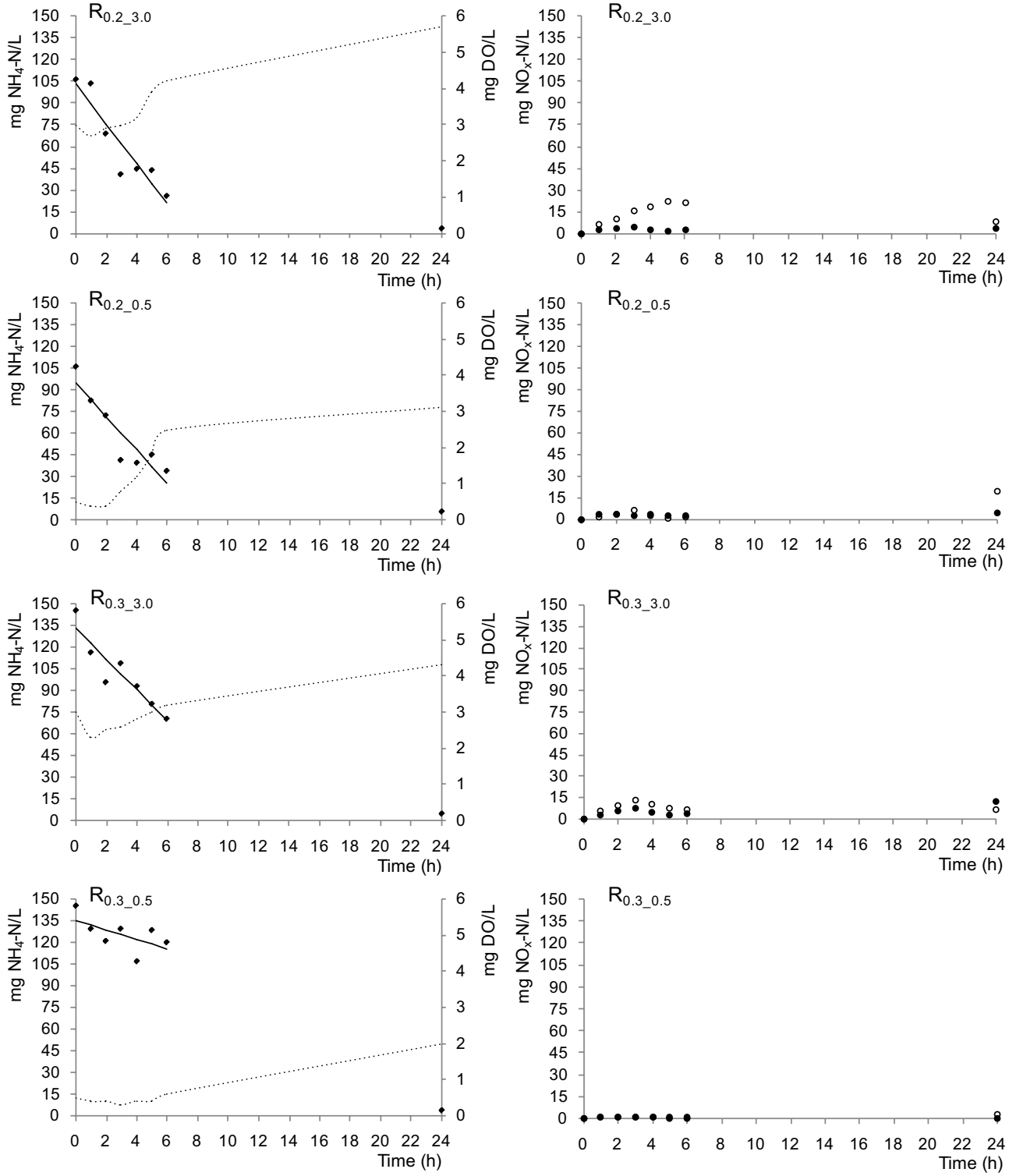

Fig. 2. Changes in ammonium and dissolved oxygen concentrations over time in stage 2 (black diamonds – ammonium concentrations, solid line – 0-order kinetics, dotted line – dissolved oxygen concentrations) and changes in nitrites (white circles) and nitrates (black circles) concentrations over time in $R_{0.2\_3.0}$, $R_{0.2\_0.5}$, $R_{0.3\_3.0}$, and $R_{0.3\_0.5}$.

91% (Table 1). This process was enabled by the anoxic zones that can exist in the very dense activated sludge that is typical in MBRs, although the flocs of MBR activated sludge are smaller than those of conventional activated sludge. Smaller floc sizes, resulting from shear forces that are created by high pressures and high turbulences from uprising air in the MBR, affect microbial growth conditions, which translates to the overall efficiency of the system [30]. In the present study, the concentration of activated sludge was about 19 g MLSS/L to maintain conditions like those that are used in the existing MBR plant from which the sludge was taken. Under such conditions, the efficiency of oxygen transfer decreases, which is why the reactors were operated under higher initial DO concentrations (2.5 and 3.0 mg DO/L) than those used in conventional activated sludge systems, in which 1.0–1.5 mg DO/L enables optimum growth of nitrifiers. Ghyoot et al. [13] found that it was possible to effectively remove nitrogen from reject water (about 90%) with a low COD/N ratio of 2.3; however, the addition of acetate or methanol was necessary. In the present study, an external carbon source was not added; therefore, it can be concluded that anoxic zones played an important role in effective denitrification. At 20% oxygen saturation in the reactor, the denitrification rate in anoxic zones was up to 40% of the rates observed under anoxic conditions; however, at 100% saturation, no denitrification occurred in activated sludge [31]. According to Fenu et al. [32], the accumulation of soluble microbial products retained by the membrane may serve as the carbon source for denitrification. Although sludge lysis could have supported denitrification in the initial phase in the present study, this will not be sustainable for long-term operation. Therefore, in the MBRs, the presence of rich and diverse microbial communities, which are active under transient aerobic and anoxic conditions, is suggested to be the most important factor that affects denitrification. For example, in the presented experiment, the presence of *Nitrobacter* sp., which due to its mixotrophic capability is able to carry out dissimilatory nitrate reduction and store PHB [33], may have favored nitrogen removal.

The highest efficiency of nitrogen removal that was obtained at the highest nitrogen loading used may have resulted from a low DO concentration of 0.5 mg DO/L. Such conditions may have favored the activity of the anammox bacteria, which accounted for 1% of the total bacteria in the activated sludge collected from the MBR. Anammox bacteria are present in wastewater treatment systems operated under high nitrogen load conditions even in reactors that are not operated in a way favoring enrichment of anammox bacteria population, for example, in reactors with constant aeration in the cycle. Mieczkowski et al. [34] have reported that anammox bacteria accounted for 4.2% ± 1.9% of biomass in constantly aerated granular reactor operated at ambient temperatures, contributing to the effective removal of nitrogen from N-rich landfill leachate. The high concentrations of ammonium in the supernatant, the absence of easily degradable COD and the partially anoxic conditions could have favored the anammox. To ensure anammox, the oxidation of nitrite needs to be inhibited, most likely by a low DO concentration, as it was in the study by Zeng et al. [22], who inhibited NOB in a continuous-flow process treating municipal wastewater by maintaining a low DO concentration (0.5 mg/L) and a short HRT (6 h). In the present study, the high nitrifying activity of AOB and inhibition of NOB was indicated by the NAR, which increased to above 0.8 in the effluent at 0.5 mg DO/L (Table 1). According to Zeng et al. [26], a NAR level > 0.8 indicates washout of

Table 1
Efficiencies of removal of organics and nitrogen compounds, and kinetic parameters of ammonium conversions and oxygen uptake

Reactor	$R_{0.1\_2.5}$	$R_{0.2\_2.5}$	$R_{0.3\_2.5}$	$R_{0.2\_3.0}$	$R_{0.2\_0.5}$	$R_{0.3\_3.0}$	$R_{0.3\_0.5}$
r_{NH_4-N} (mg/(L h))	14.8	11.8	17.0	13.7	11.6	10.6	3.3
r_{NH_4-N} (mg/(g MLVSS h))	1.0	0.8	1.2	0.9	0.8	0.7	0.2
E_{nitr} (%)	100.0 ± 4.2	99.2 ± 4.4	97.6 ± 4.8	99.3 ± 4.9	98.9 ± 4.9	99.1 ± 4.2	99.3 ± 4.3
E_{TN} (%)	87.5 ± 3.8	89.0 ± 4.2	83.2 ± 4.0	83.4 ± 4.1	74.9 ± 3.7	79.8 ± 3.8	91.5 ± 4.0
E_{COD} (%)	35.5 ± 1.8	41.1 ± 1.7	56.2 ± 2.0	5.3 ± 0.3	5.0 ± 0.5	9.5 ± 0.8	0.0 ± 0.0
NAR	0.000	0.018	0.001	0.676	0.803	0.352	0.858
k_{OURexo} (d^{-1})	0.36	0.47	0.56	–	–	–	–
OUR_{exo} (mg/(L d))	2,042.3	2,275.3	2,845.4	–	–	–	–
$SOUR_{exo}$ (mg/(g MLVSS d))	141.3	157.5	196.9	–	–	–	–
$k_{OURnitr}$ (d^{-1})	0.63	0.31	0.29	–	–	–	–
OUR_{nitr} (mg/(L d))	426.5	179.2	237.2	–	–	–	–
$SOUR_{nitr}$ (mg/(g MLVSS d))	29.5	12.4	16.4	–	–	–	–
$k_{OURendo}$ (d^{-1})	0.53	0.53	0.53	–	–	–	–
OUR_{endo} (mg/(L d))	2,678.1	2,678.1	2,678.1	–	–	–	–
$SOUR_{endo}$ (mg/(g MLVSS d))	185.3	185.3	185.3	–	–	–	–

r_{NH_4-N} – ammonium removal rate; E_{nitr} – efficiency of nitrification; E_{TN} – efficiency of total nitrogen removal; E_{COD} – efficiency of COD removal; k_{OURexo} – rate constant for oxygen uptake for exogenous respiration; OUR_{exo} – rate of oxygen uptake for exogenous respiration, $k_{OURnitr}$ – rate constant for oxygen uptake for nitrification; OUR_{nitr} – rate of oxygen uptake for nitrification; $k_{OURendo}$ – rate constant for oxygen uptake for endogenous respiration; OUR_{endo} – rate of oxygen uptake for endogenous respiration; $SOUR_{exo}$ – specific rate of oxygen uptake for exogenous respiration; $SOUR_{nitr}$ – specific rate of oxygen uptake for nitrification; $SOUR_{endo}$ – specific rate of oxygen uptake for endogenous respiration.

NOB from the system, which will ensure stable nitritation that will not be influenced by short-term changes in operational conditions. In the present study, the lower rate of ammonium removal observed at 0.5 mg DO/L than in the corresponding series with a higher DO concentration may indicate anaerobic ammonium oxidation, the rate of which is lower than that of aerobic ammonium oxidation [35]. On the other side, some nitrite accumulation at 0.5 mg DO/L, as evidenced by a NAR increase, may indicate that complete nitrification–denitrification via nitrate turned to shortcut nitrification-denitrification via nitrite as a result of low DO concentration. This may have resulted from the fact that the influent free ammonia concentration (1.5–3.7 mg/L) was not at the inhibitory level (10–150 mg/L) of AOB but was higher than the inhibitory level (0.1–1.0 mg/L) of NOB [36]. In addition, as reported by Langone et al. [3] on the treatment of supernatant of a C/N ratio of 0.5, denitrifiers can compete with anammox bacteria for nitrite, which improves the efficiency of TN removal. Therefore, when treating digester supernatants of high nitrogen and low organics contents without anammox bacteria enrichment, nitrogen is most probably removed because of the cooperation of nitrifying, denitrifying and anammox bacteria.

3.2. Removal of organic compounds

In general, MBR technology has been reported to yield high efficiencies of COD removal of up to 90% [37] or even above 95% [38] due to membrane retention and enhanced biodegradation owing to high biomass concentration and an acclimated microbial community. However, the supernatant used in the present study had a BOD_5/COD ratio of 0.2, which indicates that its biodegradability was low. Such ratios are typical of industrial wastewater and of wastewater after effective biological treatment. Thus, it can be concluded that the anaerobic stabilization of the sludge was very effective, as it produced digester supernatant with a low content of degradable organic compounds. In $R_{0.3\_2.5}$, the COD concentration dropped to 187 mg/L after 2 h of the reaction and then remained at this level resulting in a total COD removal of 56.2% (Fig. 3 and Table 1). In the other series, COD removal efficiency was much lower and COD concentrations throughout the cycle were almost stable. The lack of COD removal over time suggests that microbial lysis occurred as a result of starvation caused by the very low organic loading and long aeration time [39]. Further evidence for microbial lysis is the fact that the rate of oxygen uptake for endogenous respiration was high, 185.3 mg/(g MLVSS d) (Table 1). This high uptake indicates that in the absence of exogenous substrates oxygen was used for the oxidation of the products of lysis released from cells. No biomass growth observed during the experiment, despite of the presence of growth substrates in reject water, also indicated biomass lysis.

3.3. Respirometric activity of microorganisms

To evaluate which processes predominated in the MBR activated sludge, the oxygen uptake rates (OURs) and the specific oxygen uptake rates (SOURs) for the oxidation of organic compounds (exogenous respiration) and ammonium were measured. In an MBR design, the OUR and SOUR only indicate the current physiological status of the microorganisms to a minor extent because these indicators are a natural consequence of the very low organic loading employed [40]. In the present study, just after the beginning of the measurement, the rate of oxygen uptake for the oxidation of organic compounds reached maximum; then it decreased as organic compounds were depleted in the measuring vessel (Fig. 4). At the highest *n*, the rate of oxygen uptake for organic oxidation during the entire time of incubation was 196.9 mg/(g MLVSS d) (Table 1). At the lowest *n*, it decreased to 141.3 mg/(g MLVSS d). These values indicated good sludge activity, as Yoon [40] reported values of 48–120 mg/(g MLVSS d) as typical for MBRs.

In contrast to the oxidation of organic compounds, oxygen was not used for ammonium oxidation at the beginning of all three experiments. The lower the TN loading of the activated sludge, the longer this phase without oxygen uptake lasted. At the largest TN loading, this phase was the shortest, because more ammonium was available for oxidation. This periodic lack of oxygen uptake with simultaneous removal of ammonium (Figs. 1 and 2) may indicate that the anammox process contributed to ammonium loss. After the period of no oxygen uptake, oxygen started to be used at rates of 12.4–29.5 mg/(g MLVSS d), and the rate constant increased from 0.29 to 0.63 d^{-1} as

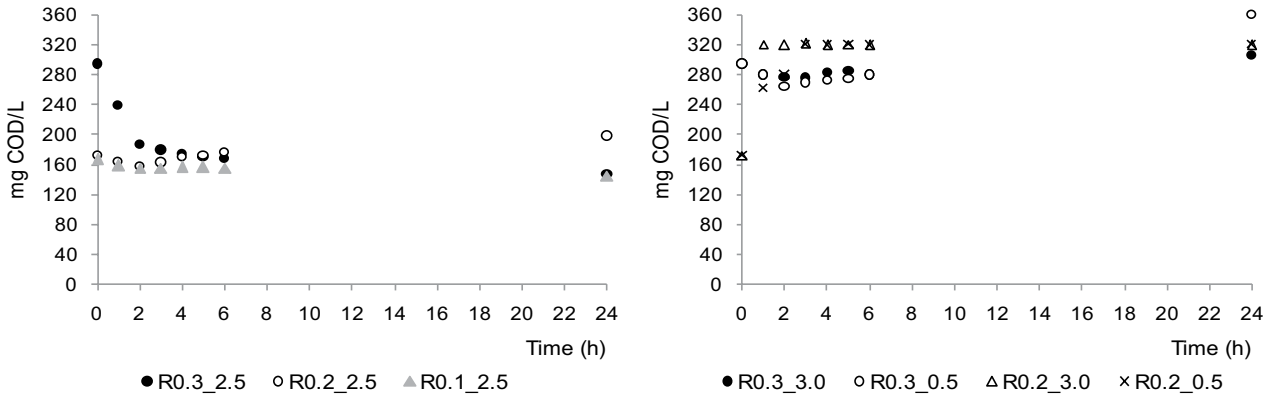

Fig. 3. Changes in chemical oxygen demand concentrations over time.

the nitrogen concentration decreased in the influent. This agreed with the increased efficiencies of nitrification from 97.6% to 99.2% and 100% under corresponding conditions in reactors $R_{0.3\_2.5}$, $R_{0.2\_2.5}$, and $R_{0.2\_2.5}$, respectively.

3.4. Microbial composition of the MBR activated sludge

The molecular analysis of the MBR activated sludge showed that, of the identified sequences, 68.9% belonged to bacteria, less than 0.01% to archaea while the other 31.1% were not identified (No Hit, Fig. 5). The sequencing depth (16,877 reads) was sufficient, as indicated by the flattening of the rarefaction curve (data not shown) as well as the nearly identical values of the observed number of operational taxonomic unit (OTUs) (309) and the Chao1 index (310.2). Taxa such as, for example, *Rhodospirillales*, *Sphingomonadales* and *Rhizobiales* that are mainly responsible for biofilm formation in MBRs and the resulting biofouling of the membranes [41] were not identified. The most numerous phyla in the activated sludge were *Bacteroidetes*, *Actinobacteria*, *Proteobacteria* and *Firmicutes*, with abundances of 29.1%, 13.4%, 11.2% and 2.2%, respectively. *Acidobacteria*, *Chlorobi* and *Chloroflexi* were less abundant (about 1.5%). Similarly, Guadie et al. [42] reported that *Proteobacteria*, *Firmicutes* and *Bacteroidetes* predominated in biomass but in an intermittently-aerated fluidized-bed reactor–membrane-bioreactor. *Bacteroidetes* were also found in biofilms on reverse osmosis membranes fed with secondary effluents from WWTPs [43]. The abundance of this phyla is desirable because its members may play an important role in the degradation of biopolymers in membranes [44].

Nitrospira sp., which predominated in a high salinity partial nitrification-anammox system [45], was the most abundant NOB detected by NGS in the present study although its average relative abundance was only 0.3%. Out of AOB, only *Nitrosomonas* sp. sequences were observed but their relative abundance among other sequences was also low (0.06%). Many taxa of denitrifiers commonly occurring in activated sludge were identified in MBR in the present study, including, for example, *Rhodocyclales*, *Comamonadaceae*, with the most abundant *Comamonas* sp., *Trichococcus* sp., which are capable of denitrification [46–48], and *Flavobacterium* sp., which are capable of aerobic denitrification [49].

Among *Bacteroidetes*, the most abundant order was *Sphingobacteriales*. Unidentified *Sphingobacteriales* constituted 9.1% of the biomass, while other identified *Sphingobacteriales* included *Terrimonas* sp., *Ferruginibacter* sp. and *Chitinophaga* sp. *Terrimonas* sp. was identified in sludge from a municipal wastewater treatment plant that struggled with sludge bulking [50], which is consistent with our results because, in this study, the biomass was also rich in filamentous microorganisms belonging mostly to *Actinobacteria* such as *Candidatus Microthrix parvicella* (4.8%). This species, together with *Haliscomenobacter hydrossis*-like bacteria and Types 0803 and 0092, were the most abundant filamentous bacteria in plants treating municipal and domestic wastewater [51,52]. Extensive growth of *Microthrix parvicella* may result in an accumulation of nitrogen while phosphorus removal is maintained because this species is able to enhance phosphate accumulation [53]. The presence of flocs with a high share of filamentous microorganisms is unfavorable because due to a loose structure of flocs no denitrification is observed even at lowered oxygen saturation (20%) in the reactor [31]. In the present study, the presence of filamentous bacteria in the MBR activated sludge did not affect the final quality of the effluent because biomass separation on the membrane was very effective, independently of sludge composition. *Ferruginibacter* sp., which constituted 4.6%

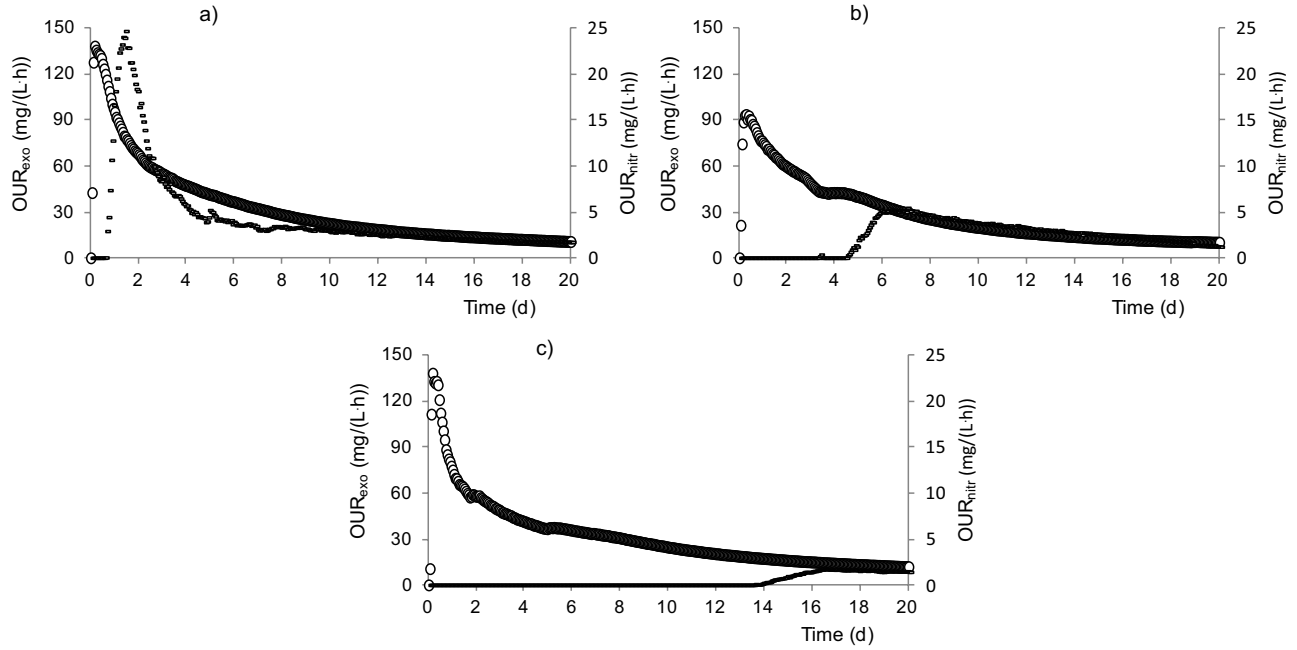

Fig. 4. Changes in OUR over time for exogenous respiration (white circles) and for nitrification (dashes); (a) n = 0.3 d^{-1}, (b) n = 0.2 d^{-1}, and (c) n = 0.1 d^{-1}.

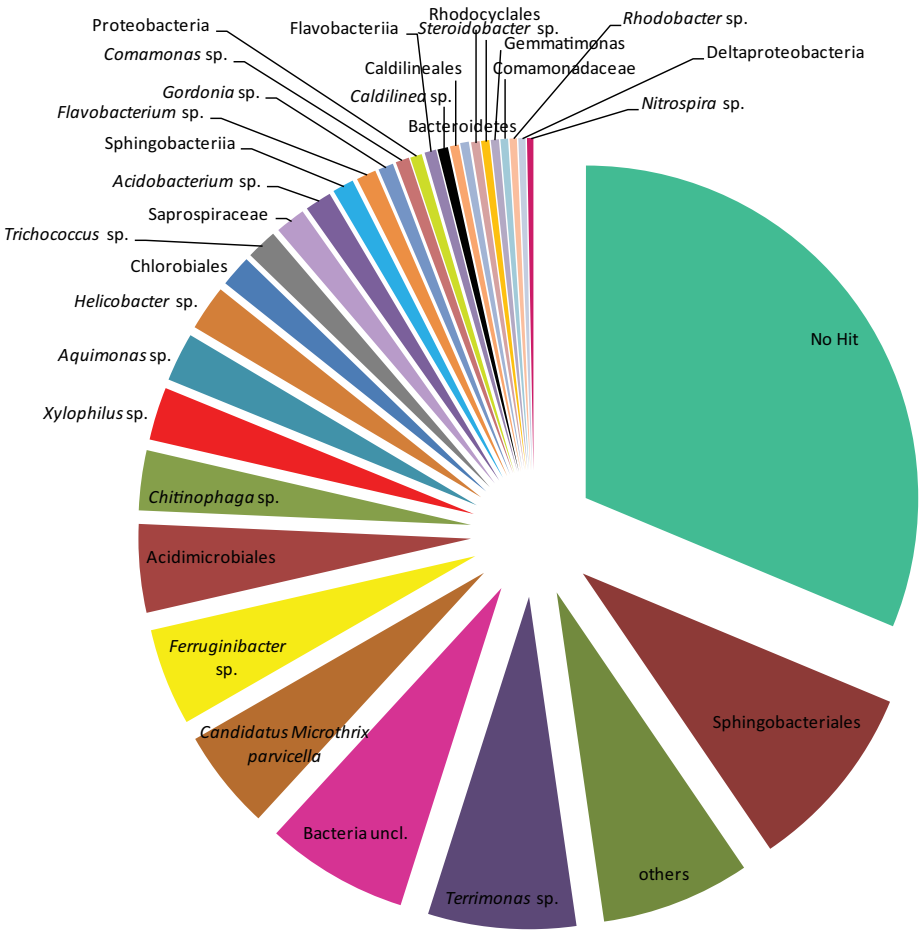

Fig. 5. Main bacterial taxa (in %) identified in the MBR activated sludge, only taxa with abundances >0.3% are presented.

of the sequences identified by NGS, was among six core genera that played a crucial role in wastewater treatment, regardless of the treatment process and geographic location [54,55]. The presence of *Ferruginibacter* sp. was desirable because they can hydrolyze organic matter [56], including difficult-to-degrade compounds [57]. Among *Proteobacteria*, the most numerous genus was *Xylophilus* sp. (2.6%), which, together with other members of the order *Burkholderiales*, was frequently retrieved from reverse osmosis biofilms [58].

4. Conclusions

In the present study, a strategy was developed that allows the use of MBR activated sludge from the main treatment line of a municipal WWTP for regular inoculation of a side-stream reactor treating nitrogen-rich digester supernatant. In the side-stream, efficient nitrogen removal was obtained because of the high biomass concentration and optimal operational parameters, despite the use of a sludge that was not adapted to high nitrogen loadings. Purification of reject water in a side-stream MBR ensures low production of external polymeric substances, which decreases the susceptibility of the membrane to fouling. However, long-term investigations on the treatment of anaerobic supernatant are necessary to determine how continuous use of the anaerobic effluent will affect the precipitation of mineral deposits on the membrane surface under alkaline conditions.

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