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# Nitrogen removal in submerged MBR with intermittent aeration

Josip Ćurko*, Marin Matošić, Helena Korajlija Jakopović, Ivan Mijatović

[a]*Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10 000 Zagreb, Croatia*
*Tel. +38514605026; fax +38514605072; email: jcurko@pbf.hr, Tel. +38514605027; fax +38514605072; email: mmatosic@pbf.hr, Tel. +38514605027; fax +38514605072; email: hkorajlija@pbf.hr, Tel. +38514605131; fax +38514605072; email: imijat@pbf.hr*

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A B S T R A C T

For this paper, synthetic wastewater was treated in two membrane bioreactors, with an accent on the removal of total nitrogen through nitrification and denitrification processes conducted as a batch process and as a process with continuous feed inflow. In the first bioreactor, with the flat sheet membrane, the specific denitrification rate during the batch process was 1.83 mg $NO_3^-$-N/g MLSS h, with the glucose added all at once at the start of the process. When the glucose was being added continuously, specific denitrification rate was 1.2 mg $NO_3^-$-N/g MLSS h. Without added glucose, specific denitrification rate was 0.4 mg $NO_3^-$-N/g MLSS h. Nitrogen removal was conducted with continuous feed inflow and with intermittent aeration with a different duration of aeration and non-aeration phases. The best results in the experiment were achieved when the aeration regime was set to 60 minutes aeration and 120 minutes without aeration, resulting in the reduction of total nitrogen from 45 mg/L to about 12 mg/L. In the second bioreactor, with a hollow fibre membrane, specific nitrification rate during batch nitrification amounted to 1.21 to 1.48 $NO_3^-$-N /g MLSS h. When all of the glucose was added at the start of the experiment, the specific denitrification rate ranged from 2.75 to 3.15 $NO_3^-$-N/g MLSS h. The best nitrogen removal in the experiment, amounting to 90%, was achieved with a continuous feed inflow and with the intermittent aeration regime set to 60 minutes of aeration and 120 minutes without aeration, with a glucose concentration in feed water of 0.72 g/L.

***Keywords:*** MBR; Nitrification; Denitrification; Intermittent aeration

## 1. Introduction

There is an increasing demand for better quality effluents from wastewater treatments. Since conventional activated sludge treatment (ASP) in some cases cannot cope with either the composition of wastewater or the fluctuations in wastewater flow rate, a promising alternative, the membrane bioreactor (MBR) technology, which combines biological ASP and membrane filtration, has became more popular in recent years. With the increasing pressure on water resources worldwide, there is a need to consider recycling and reuse of wastewater effluents, which has also brought MBR in focus when demand for effluent quality exceeds the capability of ASP [1,2]. Because of their good qualities, MBRs are rapidly gaining in popularity, and are a promising technology for present and future wastewater treatments [1]. Use of MBRs with submerged membranes has several advantages over conventional ASP, including stability and high effluent quality, ease of operation, small footprint, and absolute removal of bacteria because MBR uses membrane filtration instead of sedimentation to separate bacteria from the treated water. In addition, without the need

*Corresponding author

Table 1
Characteristics of submerged membranes

| | MBR 1 | MBR 2 |
|---|---|---|
| Membrane brand | Kubota, XJ3 | Zenon, ZW-10 |
| Membrane type | Flat sheet | Hollow fibre |
| Membrane dimensions | 226 mm × 316 mm × 6 mm | 692.15 mm × 109.54 mm |
| Pore size | 0.4 μm | 0.4 μm |
| Membrane area | 0.33 $m^2$ | 0.93 $m^2$ |

for the settling ability of sludge, biomass concentration within the bioreactor can be maintained at a much higher level, thus reducing the size of the bioreactor. Sludge retention times (SRT) are in general much longer with a MBR, which gives the slower growing species, which have the ability to decompose less biodegradable compounds, the opportunity to proliferate. A drawback of MBR use is the need for intensive aeration, as well as membrane fouling and the related need for membrane cleaning and replacement.

Nitrogen removal by biologic nitrification and denitrification with conventional ASPs is often difficult because of insufficient concentration of nitrificating autotrophic microorganisms, which are flushed out of the aeration pools at low SRT values common for ASP because of their slow growth rate. MBR, with a much greater SRT, is capable of much more stable nitrification. However, good nitrification must be followed by successful denitrification for total nitrogen removal. Anoxic conditions needed for denitrification have to be separated from aerobic conditions needed for nitrification either by physically separating processes through the installation of a separate anoxic tank [3–5], or by separating the two processes in time in one bioreactor [6,7]. The first approach increases the footprint of the treatment plant while the second increases problems with fluctuations of nitrogen species concentration in the effluent, which are sometimes attempted to be solved by discontinuation of the flow rate, which then increases hydraulic retention time and decreases the volume efficiency of the process. Kimura et al. [8] tried to rectify that problem by installing a partition into the very reactor where nitrification and denitrification were performed. When nitrification and denitrification are performed in the same bioreactor, there arises the problem of membrane fouling, which is prevented by turbulent airflow, but which is not present during the anoxic stage of denitrification. In this paper, during the anoxic part of the experiment, active sludge suspension was circulated along the membrane by a pump, which should have prevented the clogging, and at the same time allowed the exchange of nutrients in the floccules of activated sludge.

## 2. Methods

### 2.1. Experimental design of MBRs

In this paper, two pilot plants were used for wastewater processing. The first pilot plant (MBR 1) constituted of a bioreactor with a total volume of 31 L (14 × 22 × 103 cm), in which there was a flat sheet membrane, a XJ3 module by Kubota (Table 1), a vacuum meter, a compressor for air supply and a diffuser at the centre of the bioreactor, a timer switch, an air flow meter, a piston pump, which was used to pump synthetic wastewater into the bioreactor and pump the processed water out of the reactor, as well as a centrifugal pump used to stir sludge during wastewater treatment without aeration. The 20 L of activated sludge used, was brought from the municipal wastewater treatment plant. The bioreactor was aerated with a constant airflow of 0.9 $m^3$/h, which produced a high dissolved oxygen concentration, always above 6 mg/L, except during periods of denitrification when the aerator was turned off.

The other pilot plant (MBR 2) was of similar design as MBR 1, with a 54 L bioreactor (24 × 24 × 93 cm) and with a hollow fibre membrane by Zenon, module ZW-10 (Table 1). It consisted of an air supply compressor, thermometers, vacuum meters and airflow meters. Synthetic wastewater was brought in and out of the bioreactor using a piston pump. A centrifugal pump was used to stir the sludge when the aeration stopped. At the centre of the bioreactor, there was an aeration diffuser at the lower part of the casing, through which air was supplied using the compressor at a rate of 3.4 $m^3$/h during the aeration phases of the experiment. The treated water was drawn from the centre of the submerged membrane, using vacuum created by the piston pump. The 40 L of activated sludge from the municipal wastewater treatment plant was used for the MBR 2.

### 2.2. Activated sludge

Two types of activated sludge were used in the experiment. In MBR 1, 20 L of activated sludge was

Table 2
Composition of synthetic wastewater

| Substance | Concentration (g/L) |
|---|---|
| Glucose | 0.180 |
| Peptone | 0.0857 |
| Yeast extract | 0.00116 |
| $NH_4Cl$ | 0.2516 |
| $KH_2PO_4$ | 0.0179 |
| $MgCl_2.4H_2O$ | 0.002 |
| $FeCl_3.6H_2O$ | 0.00011 |

used (Activated Sludge A). It was brought from the municipal wastewater treatment plant of the town of Velika Gorica, Croatia, and was cultivated for three months with synthetic wastewater at aerobic conditions. And a week before the experiments listed in Tables 3–5, it was adapted to aerobic and anaerobic conditions by turning the aerator on and off with a timer switch every 60 min. The synthetic wastewater during this cultivation was the same and it is given in Table 2.

In MBR 2, 40 L of activated sludge (Activated Sludge B) from the municipal wastewater treatment plant of the city of Čakovec, Croatia, was used for inoculation. The acclimatisation of the activated sludge for the experiment took two weeks with the same synthetic wastewater as for MBR 1. In the first week, with constant aeration in order to adapt the sludge for work with synthetic wastewater, and in the second week with alternating aerobic and anaerobic conditions in order to develop the denitrification ability in the same manner as with Activated Sludge A.

During the conducting of the experiment, the only loss of sludge occurred during the taking of the samples for the determination of MLSS, so the age of the sludge can be estimated at 150 days. In both MBRs, the MLSS stabilized at about 5 g/L in prolonged cultivation.

### 2.3. Synthetic wastewater

Synthetic wastewater used in the experiment was prepared daily in the laboratory. Synthetic water was prepared in a 350 L barrel, by dissolving set concentrations of substances in a certain volume of water. These substances were glucose, peptone, yeast extract, ammonium chloride ($NH_4Cl$), monopotassium phosphate ($KH_2PO_4$), manganese chloride ($MnCl_2{\cdot}4H_2O$) and ferric (III)-chloride ($FeCl_3{\cdot}6H_2O$). The composition of the model wastewaters with appropriate concentrations is shown in Table 2. Synthetic wastewater was supplied to the bioreactor MBR 1 using a piston pump, at a flow rate of 1.8 L/h, and this flow rate never changed during the experiment, with the retention time of 11 h. Synthetic wastewater was supplied to MBR 2 at flow rate of 4 L/h, with no change and with a retention time of 11 h.

### 2.4. Batch nitrification

The batch nitrification experiment was conducted in both MBRs. Before the start of the experiment, the aerator was set using the timer switch, so that during the night, it aerated for 60 min and was switched off for 120 min. Eight hours before the start of the experiment and taking the first sample, the aerator was switched off, so that the nitrate concentration would drop to 0 mg/L because of biological denitrification. Then the aeration was turned on and the concentration of nitrates, nitrites and pH was monitored. Samples were taken every 30–50 min. The specific nitrification rate (SNR) was calculated using the direction coefficient of the linear first part of nitrate concentration-in-time curve divided by MLSS. During the experiment, N2, $NaHCO_3$ was being added to MBR 2 in batches (7.5 g in the 140th minute; 5 g in the 215th minute and 2.5 g in the 245th minute of the experiment), for the correction of pH, because a greater concentration of incoming nitrogen was used in the form of ammonium chloride (5.3 g), which was added at the start of the experiment itself.

### 2.5. Batch denitrification

The experiment was conducted in both MBRs with different ways of adding glucose into the bioreactor, so that glucose was added either at the start, continuously during denitrification, in batches or was not added at all. A summary of different experimental conditions is shown in Table 3. Aeration was turned on for eight hours before the start of the experiments (D1 to D7), and during the preceding 18 h, one hour of aeration had been alternated with an hour without aeration using the timer switch in experiments D8 to D10. During the experiment, the pump used to supply synthetic wastewater to the bioreactor and drain the purified water out of the bioreactor was switched off. The samples were taken by turning on the pump for short instances. Effluent samples were taken every 30 to 90 minutes during the 4 to 6 hour duration of the experiment. Nitrate concentration and pH values of the samples were determined. Before the conducting of denitrification, the time needed to achieve anaerobic conditions was set. Specific denitrification rate (SDR) was calculated using the direction coefficient of the linear first part of the nitrate concentration-in-time curve divided by MLSS.

Table 3
Different experimental conditions in batch denitrification

| Experiment | MBR | Mode | Aeration regime | Glucose addition | Aeration regime before experiment |
|---|---|---|---|---|---|
| DN1 | 1 | Batch | NA | 0.5 g every 30 minutes | 8 h A |
| DN2 | | | | Constant addition of 0.33 g /h | 8 h A |
| DN3 | | | | None | 8 h A |
| DN4 | | | | 4 g at the beginning | 8 h A |
| DN5 | 2 | | NA | 11 g glucose at the beginning | 8 h A |
| DN6 | | | | 11 g glucose at the beginning | 8 h A |
| DN7 | | | | None | 8 h A |
| DN8 | | | | 11 g glucose at the beginning | 1 h A and 1 h NA |
| DN9 | | | | 11 g glucose at the beginning | 1 h A and 1 h NA |
| DN10 | | | | 5 g glucose at the beginning and 1 g glucose every 40 min | 1 h A and 1 h NA |

A – aeration; NA – no aeration.

### 2.6. Continuous nitrogen removal

Continuous nitrogen removal was performed by repeated nitrification and denitrification in both MBRs, and a summary of different experimental conditions is shown in Table 4. In the experiment C1 in MBR 1, 0.4 g of glucose (20 mg glucose per L of biomass suspension) was added at the start of the denitrification stage, and in the others, denitrification was performed only with the flow of synthetic wastewater. In the experiments in MBR 2 (C4, C5 and C6), the concentration of glucose in incoming synthetic water was 0.18, 0.36 and 0.72 g/L, respectively. The duration of the denitrification stage in the experiments alternated between 60 and 120 min. The nitrification stage always lasted 60 min. Samples were taken every 30 to 50 minutes during the 4 to 6 hours of the experiment, and the concentration of nitrates, nitrites and ammonia, total nitrogen, pH and TOC were determined.

### 2.7. Respiratory activity of activated sludge

Four measurements of respiratory activity were taken in both Activated Sludge A and Activated Sludge B; two experiments without adding glucose into the bioreactor and two experiments with the addition of 4 and 10 g of glucose (glucose was added before switching the aeration off). The aeration was first switched on in order to achieve a high concentration of dissolved oxygen in the activated sludge with a constant supply of wastewater during several hours. Before inserting the oxygen measurement electrode, the aeration and synthetic water supply were turned off. The electrode was put into the bioreactor while stirring the water around the electrode, and the decrease of concentration of oxygen was then measured in 30-second intervals. The specific respiration rate was determined by dividing the coefficient of oxygen concentration vs. time curve with the biomass concentration (MLSS).

Table 4
Different experimental conditions in continuous nitrogen removal

| Experiment | MBR | Mode | Aeration regime | Feeding regime | Aeration regime before experiment |
|---|---|---|---|---|---|
| C1 | 1 | continuous | 1 h A<br>1 h NA | Synthetic wastewater + 0.4 g of glucose at the start of every NA stage | 8 h NA |
| C2 | | | 1 h A<br>1 h NA | Synthetic wastewater | 1 h A and 1 h NA for 24 h |
| C3 | | | 1 h A<br>2 h NA | Synthetic wastewater | 1 h A and 1 h NA for 18 h |
| C4 | 2 | | 1 h A<br>2 h NA | Synthetic wastewater | 1 h A and 1 h NA for 18 h |
| C5 | | | 1 h A<br>2 h NA | Synthetic wastewater with 0.36 g/L glucose | 1 h A and 1 h NA for 18 h |
| C6 | | | 1 h A<br>2 h NA | Synthetic wastewater with 0.72 g/L glucose | 1 h A and 1 h NA for 18 h |

A – aeration; NA – no aeration.

Table 5
SDR for MBR 1

| SDR mg $NO_3^-$-N/ g MLSS h | Description |
|---|---|
| 1.2 | DN1 – Addition of 0.5 g glucose every 30 minutes |
| 1.52 | DN2 – Constant addition of 0.6 g/L glucose with 1.8 L/h |
| 0.4 | DN3 – Without glucose |
| 1.83 | DN4 – Addition of 4 g glucose at the beginning |

### 2.8. Analytic methods

Total organic carbon was determined using the TOC-5000A TOC analyser made by Shimadzu. Oxygen concentration was determined using the WTW 330i oximeter. Other analyses were performed according to standard methods.

## 3. Results and discussion

### 3.1. Nitrification

One of the advantages of MBR over classic technology is its ability to keep slowly growing microorganisms in the bioreactor, since it can operate with much older sludge with high SRT. That is especially important in the case of nitrificating bacteria, which grow slowly, and are very important for the removal of nitrogen from wastewater. In conventional ASP, the flushing out of nitrificating microorganisms and nitrification faults can often occur at low SRTs. That is why batch nitrification experiments were conducted, after adapting the sludge to synthetic wastewater, in order to measure the nitrification capability of the activated sludge. In order to observe the nitrification capability, the level of nitrates was decreased to 0 mg/L, which was achieved by turning the aeration off 8 hours before the start of the experiment, in order to remove the nitrates through denitrification.

Nitrification was performed equally successful in both MBRs. During the conducting of the experiment N1 in MBR 1, nitrate concentration and pH were monitored. Nitrate concentration at the end of the experiment amounted to 23 mg/L, and the SNR was calculated from the linear part of the curve (0 to 17.4 mg/L of nitrates) and amounted to 1.94 mg $NO_3^-$-N/g MLSS h, which confirms the good nitrification capability of Activated Sludge A. The resulting value of SNR is double the resulting SNR for similarly aged sludge in the work of Han et al. [9], who gave SNR for different ages of sludge. Such a discrepancy in the results is probably caused by the much greater sludge concentrations at which they worked, so it is possible that they had a lower oxygen transfer rate within the floccules of activated sludge. Measured pH values of 7.53–7.93 indicate a very slight change in pH, as a consequence of nitrification [10]. Such results were caused by a relatively low nitrogen concentration in the synthetic wastewater and sufficiently high alkalinity, which acted as a buffer and prevented the decrease of pH.

In the experiment N2 conducted in MBR 2, when 5.3 g of ammonium chloride was added as an additional source of nitrogen, the pH value dropped from 6.93 to 5.5, while the nitrate concentration increased to 30 mg/L. The decrease in pH values was corrected by adding $NaHCO_3$. The specific nitrification rate was 1.48 mg $NO_3^-$-N/g MLSS h, which also confirms the good nitrification capability of the activated sludge. Adding of additional source of nitrogen in this experiment was conducted to observe nitrification ability of activated sludge when it was faced with higher amount of nitrogen. A number of authors have researched efficiency and rate of nitrification in MBR. In the paper by Panswad and Polprucksa [11], SNR of an activated sludge process treating two synthetic wastewaters with the addition of zinc was measured. Wastewaters had nitrogen concentrations of 40 and 175 mg $NH_4^+$-N/L. In steady state (when no zinc was added) and for synthetic wastewater with 500 mg/L COD and 40 mg/L of $NH_4^+$-N, SRN amounted to 4.0 mg $NH_4^+$-N/g MLSS h. SNR was lower in our study probably due to the lower relative ratio of nitrifiers in the activated sludge. In the case of wastewater with 3500 mg/L COD and 175 mg $NH_4^+$-N/L, these authors achieved SNR of 1.5 mg $NH_4^+$-N/g MLSS h which is similar to the rates we obtained. De Silva et al. [12] determined the parameters for a mathematical model of removal of total nitrogen and COD during aeration and without aeration. Zhang et al. [13] compared water treatment in a sequencing batch MBR and a conventional MBR. They achieved a good nitrification activity in both cases, amounting to 0.56 and 0.40 mmol $NH_4^+$-N/ (g VSS day) (approximately 0.26 and 0.19 mg $NH_4^+$-N/g MLSS h) for the conventional and sequencing batch reactors, respectively, but the sequencing batch reactor had a more stable efficiency of nitrogen removal at different influent COD/TN ratios. Both SNRs measured by them were lower than our SNRs. Li et al. [14] monitored nitrification performance and microbial community dynamics in MBR treating completely inorganic wastewater, where nitrification was almost completely obtained at a volumetric loading rate of 1.2 g $NH_4^+$-N/L $day^{-1}$ with

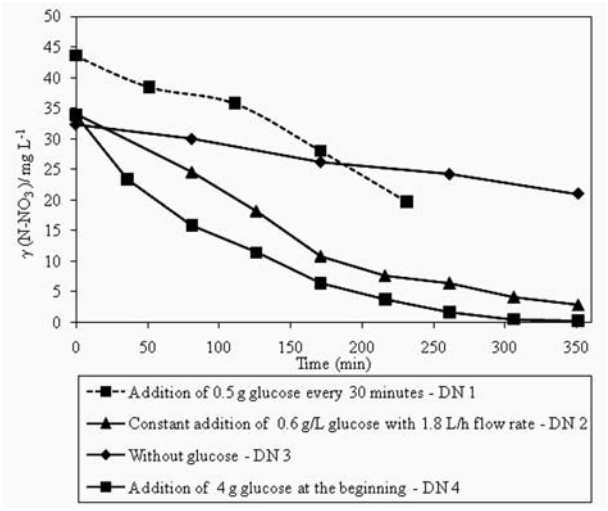


Fig. 1. Denitrification in MBR 1.

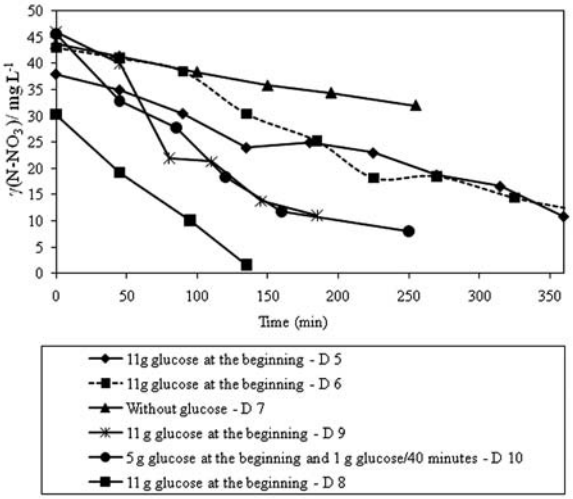


Fig. 2. Denitrification in MBR 2.

a very high SNR, ranging from 7 to 16 mg $NO_3^-$-N/g MLSS h. They reported a gradual decrease in nitrification activity during their long-term experiment and attributed the decrease to inert materials accumulation in the MBR under a long SRT caused by the complete sludge retention. It can be concluded that SNR depends on the relative ratio of nitrifiers in the activated sludge, which is in most cases a function of SRT or a function of the carbon to nitrogen ratio in the wastewater.

### 3.2. Denitrification

After measuring the nitrification activity of the activated sludge, denitrification activity measurement experiments were conducted. The experiments consisted of batch removal of nitrates produced by biologic nitrification turning off aeration and the achievement of anaerobic conditions inside the bioreactor. The denitrification rate measurement was conducted with different ways of adding an external carbon source in the form of glucose. The results of the experiment are shown in Figures 1 and 2.

In the first experiment DN1, 0.5 g of glucose was added into the MBR 1 every 30 minutes during 230 minutes, so that a total of 4 g of glucose or 0.2 g of glucose per litre of active sludge suspension was added. The starting nitrate concentration of 43.6 mg/L was decreased during 230 min to 19.7 mg/L, with a roughly constant denitrification rate, which did not decrease at the end of the experiment. So that it can be assumed that the nitrate concentration would have continued to decrease had the experiment been continued. In the next experiment, DN2, glucose was added constantly with a flow of 1.8 L/h during 6 hours, and the added solution had a concentration of 0.6 g/L, so that a total of 6.5 g of glucose was added into the bioreactor. The starting nitrate concentration was 34 mg/L and the final 2.8 mg/L. The denitrification rate was faster in the first part of the experiment and decreased with the disappearance of nitrates. The measured TOC concentrations in the effluent during the experiment indicate that, at the start of the batch denitrification experiment, the source of carbon was used for denitrification (TOC concentrations in the effluent below 10 mg/L), while near the end of the denitrification process in the 200th minute, TOC concentration increased and reached 35 mg/L in the 350th minute. This was probably caused by the decrease in the concentration of electron acceptors in the form of nitrates for the conducting of the respiratory cycle. That is why the microorganisms utilized the constantly inflowing glucose more slowly, which resulted in a TOC increase in the effluent near the end of the experiment.

No glucose was added in the third experiment DN3; and the starting nitrate concentration was lowered from 32.4 mg/L, to 21 mg/L during 6 hours. Poor denitrification activity was obviously caused by the lack of organic substrate for the conducting of denitrification, and the noted drop in nitrate concentration can be attributed to the substrate remaining in the bioreactor from the period before the start of the experiment, or to the substrate in the form of polymers in the cells.

In the last experiment, DN4, 4 g of glucose (0.2 mg/L) was added at the very start of the experiment. Nitrate concentration dropped form the starting value of 33.9 mg/L to the final value of 0.3 mg/L at the end of the experiment after 6 hours, when the decrease

Table 6
SDR in MBR 2 (MLSS 3.9–4.9 g/L)

| SDR mg $NO_3^-$-N/gMLSS h | Description |
|---|---|
| 1.55 | DN5 - After constant aeration with addition of 11g glucose at the beginning |
| 1.69 | DN6 – After constant aeration with addition of 11 g glucose at the beginning |
| 0.7 | DN7 – After growth with constant aeration and without glucose |
| 3.15 | DN8 – After growth 1 h A+1 h NA with addition of 11 g glucose at the beginning |
| 2.75 | DN9 – After growth 1 h A+1 h NA with addition of 11 g glucose at the beginning |
| 2.53 | DN10 – After growth 1 h A+1 h NA and with addition of 5 g glucose at the beginning and 1 g glucose every 40 minutes |

A – aeration; NA – nonareation.

of the denitrification rate was again visible parallel to the disappearance of the nitrates. TOC concentration experienced a sharp leap to 35 mg/L at the start of the experiment, which was caused by adding glucose, after which the concentration dropped to about 5 mg/L in the 90th minute of the experiment.

In all of the experiments, the pH value of the activated sludge suspension did not significantly change, although it is known that an increase of pH value occurs during denitrification [10]. The reason for that was the low concentration of nitrate nitrogen in the bioreactor.

In the work by Yang et al. [15], where granulated activated sludge was used in a batch bioreactor, the complete removal of nitrates, from the starting concentration of 25 mg/L of nitrate nitrogen, was achieved already after 120 min And the reason for the better performance of denitrification is possibly the addition of a three times greater quantity of external carbons sources, a somewhat lower starting nitrate concentration and a greater biomass concentration (above 10 g/L).

SDRs shown in Table 5 are based on the curves from Fig. 1. Resulting figures confirm that the external carbon source is critical for the denitrification rate, because SDR in the experiment DN3 was 0.4 mg $NO_3^-$-N/g MLSS h when no glucose was added, which is three to four times slower than the experiments where glucose was added. Furthermore, it was noted that the way of adding glucose and the quantity of added glucose have an effect on the SDR. From the stochiometric reaction of denitrification, it can be calculated that about 2.7 g of glucose is needed to remove 1 g of nitrates [10], and in the experiment, twice the theoretically necessary quantity of glucose was added. By adding glucose in batches (DN1), a SDR of 1.2 mg $NO_3^-$-N/g MLSS h was achieved. When glucose was being added constantly (DN2), SDR amounted to 1.52 mg $NO_3^-$-N /g MLSS h, and when glucose was added at the start of the experiment DN4, SDR amounted to 1.83 mg $NO_3^-$-N /g MLSS h. From the results, it is visible that the specific denitrification rate was the greatest when glucose was added at the start of the experiment. In the work of Han et al. [9], they got a somewhat lower SDR than in this experiment. The reason for this can be sought in the different methods of carbon source addition, a higher activated sludge concentration in their work, as well as the ever-present differences in activated sludge composition.

Six batch denitrification experiments shown in Fig. 2 are conducted in MBR 2, and are used to calculate SDR shown in Table 6.

Four denitrification experiments (DN5, DN6, DN8 and DN9) were conducted so that 11 g of glucose was added into the bioreactor at the start of the experiment (glucose concentration in the bioreactor 0.275 g/L), with starting nitrate concentrations of 30, 38, 43 and 46 mg/L. After that, the experiment was conducted without adding glucose as an external carbon source (DN7). Nitrate concentration was decreased from 43.8 to 19.8 mg/L, and the lowest specific denitrification rate of 0.7 mg $NO_3^-$-N/g MLSS h was recorded here. The greatest SDR was recorded in the experiment DN5 with 30 mg/L, which was the lowest starting nitrate concentration, with the nitrate concentration dropping almost to zero in 130 min. Finally, experiment DN10 was conducted, where glucose was added in batches (5 g of glucose at the start, and then 1 g of glucose every 40 minutes). Nitrate concentration decreased from 45.6 to 8 mg/L. The denitrification rate recorded here was somewhat lower than in the previous experiments, similar to the experiments with MBR 1. In all six denitrification experiments, a slight drop in pH values of approximately 0.5 pH units was recorded in the first hour, followed by an increase in pH values (approximately 0.5 to 1 pH units, depending on the nitrate concentration at the start of the experiment). An increase of pH values is expected during denitrification [10], and the initial drop in pH is probably caused by the better dissolution of $CO_2$ produced by metabolic processes of microorganisms of the activated sludge after the cessation of aeration.

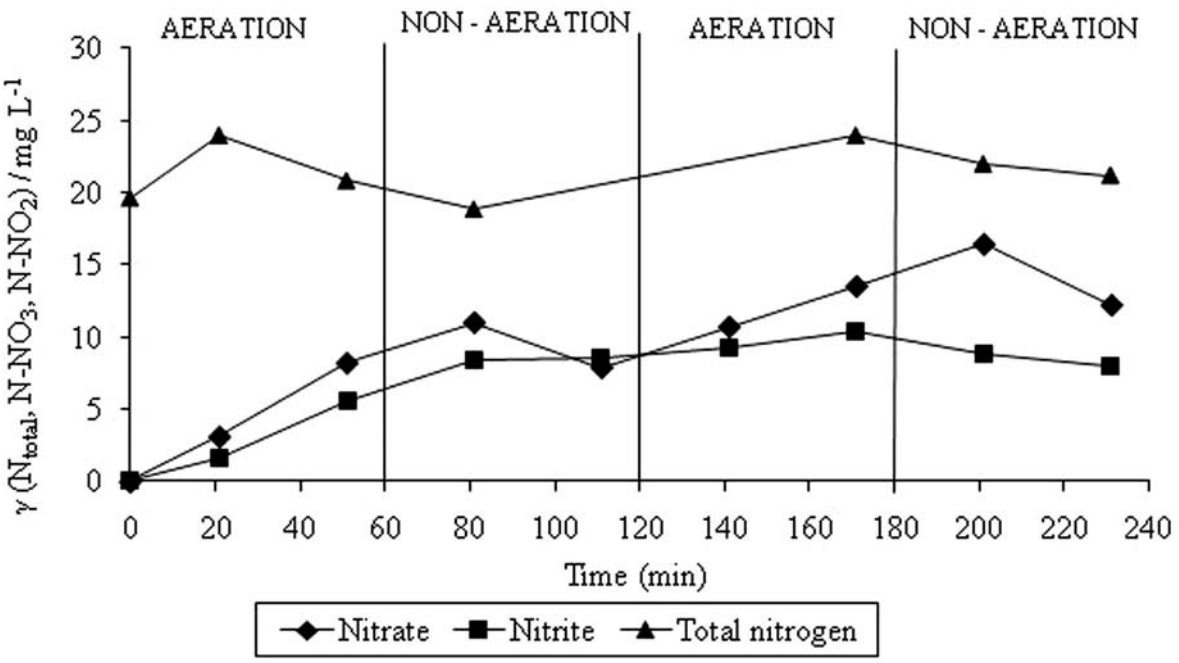


Fig. 3. Continuous treatment of synthetic wastewater in MBR 1 (C1) with intermittent aeration (60 minutes aeration and 60 minutes non-aeration) after 8 h of anaerobic conditions.

*3.3. Continuous removal of nitrogen*

After batch nitrification and denitrification experiments, experiments were conducted with continuous inflow of synthetic wastewater and with different durations of aerobic and anaerobic conditions. Three experiments (C1, C2, and C3) were conducted in MBR 1.

In the experiment C1 (Fig. 3), the concentrations of nitrates, nitrites and total nitrogen were measured during the continuous process of nitrification and denitrification with the addition of 0.4 g of glucose at the start of every anaerobic stage. The aerobic conditions lasted for 60 min and the anaerobic for 60 min. Before the start of the experiment, the aeration was turned off for 8 h, and anaerobic conditions were created, with a constant flow of synthetic wastewater into the bioreactor and constant flow of treated water through the membrane out of the bioreactor. Such conditions were set in order for the nitrate and nitrite concentrations in the bioreactor to drop to 0 mg/L through biologic denitrification. The figure shows the fluctuation of the concentration of total nitrogen from 18.8 to 24 mg/L, and that a satisfactory removal of total nitrogen from the synthetic wastewater was not achieved. The good nitrification capability of the sludge during aeration can be seen in the increase of nitrate and nitrite concentrations. During the anaerobic stage, the concentration of nitrates, nitrites and total nitrogen did not decrease. Therefore, the denitrification did not remove a sufficient quantity of nitrates, which was caused by the short duration of anaerobic conditions, under which denitrification was conducted. In addition, the concentration of nitrates and nitrites was increasing even when the aeration was turned off, because a period of 5-10 min was needed to achieve anaerobic conditions in the bioreactor, during which nitrification continued. The start of denitrification activity was much slower, because the heterotrophic microorganisms needed a certain time to adapt to the change from glucose oxidation by oxygen to oxidation by nitrate.

In the experiment C2, the regime of turning the aeration on and off remained the same (60 min of aeration, 60 min without aeration) during 24 h, with a constant supply of synthetic wastewater into the bioreactor, after which the concentration of nitrites, nitrates and total nitrogen in the effluent was measured again. Because of the continuous regime of turning the aeration on and off during 24 h, both nitrites and nitrates were present in the outgoing water. As opposed to the preceding experiment C1 (Fig. 3), no glucose was added at the start of the denitrification stage. The results of this experiment, in which nitrate, nitrite, total nitrogen and TOC concentrations were monitored during six hours, are shown in Fig. 4. The nitrite concentration was low, while the nitrate concentration was high (about 20 mg/L) but somewhat lower than the concentration of total nitrogen, which means

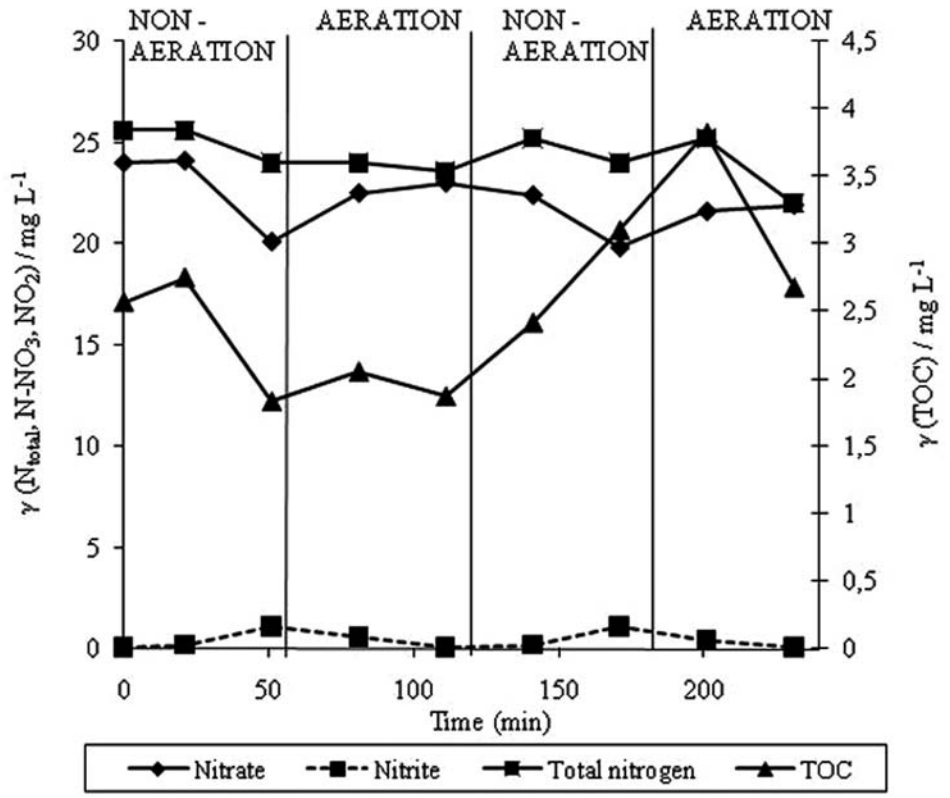


Fig. 4. Continuous treatment of synthetic wastewater in MBR 1 (C2) with intermittent aeration (60 minutes aeration and 60 minutes non-aeration).

that the nitrification process progressed well, but the denitrification process again failed to remove nitrates. TOC concentration was low and ranged from 3.8 to 1.8 mg/L, proving good removal of organic matter. The reason for the poor performance of the denitrification process was again the too short duration of anaerobic conditions, under which denitrification occurs. The same happend in the work of Lim et al. [16], where they recorded the poorest removal of nitrogen during the shortest duration of the denitrification stage. It is obvious that the heterotrophic bacteria were not able to adapt to anaerobic conditions and remove the nitrates created during the aerobic stage within the allowed time of 1 hour.

In the experiment C3, non-aeration time was increased to 120 min, with the same aeration time (60 min). The results of the experiment of continuous nitrification and denitrification, in which nitrate, nitrite, total nitrogen and TOC concentrations were monitored, are shown in Fig. 5. The results indicate good nitrification and denitrification capability of activated sludge for the chosen regime. The nitrite concentration was low, and increased only at the start of the nitrification, which has already been noted with batch nitrification. Total nitrogen concentration dropped up to four times from the concentration in the incoming water, but increased during nitrification when there was no nitrogen removal through denitrification, with only ammonium nitrogen being converted into nitrate nitrogen. Such changes of concentration of total nitrogen are usually not seen in classic wastewater processing, because of additional denitrification in the secondary sedimentation tank, where the retention time can be up to several hours.

Three continuous nitrification and denitrification experiments (C4, C5, and C6) were also conducted in MBR 2. In all the experiments aerobic and anaerobic conditions alternated during 18 hours before the start of the experiment, with one hour of aeration and two hours without aeration.

In the experiment C4, the glucose concentration in incoming water was 0.18 g/L (Fig. 6). The bioreactor was aerated for 60 min, while the period without aeration amounted to 120 min. One should keep in mind that the aerobic conditions (more than 1 mg $O_2$/L) lasted about 70 min, while the anaerobic conditions lasted about 110 min, because of the time needed for the concentration of dissolved oxygen in the bioreactor to decrease to 0 mg/L. Nitrite concentration was low

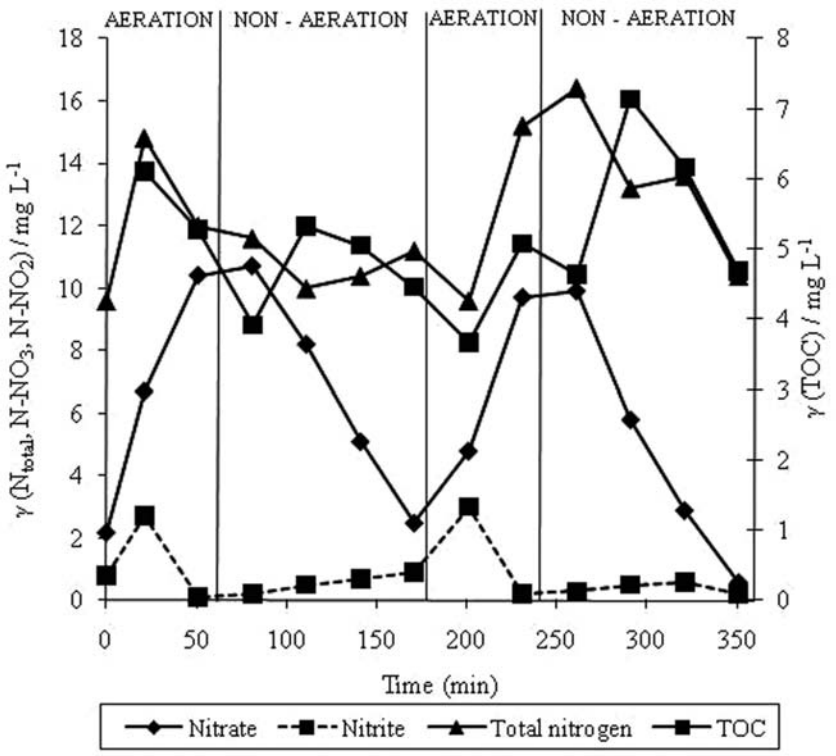


Fig. 5. Continuous treatment of synthetic wastewater in MBR 1 (C3) with intermittent aeration (60 minutes aeration and 120 minutes non-aeration).

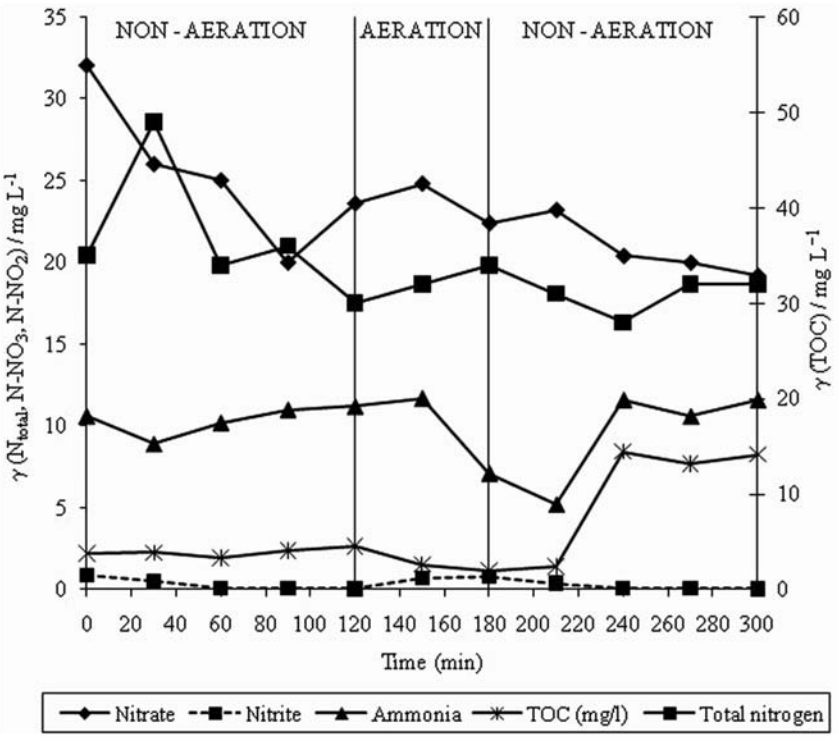


Fig. 6. Continuous treatment of synthetic wastewater containing 0.18 g/L of glucose in MBR 2 (C4) with intermittent aeration (60 minutes aeration and 120 minutes non-aeration).

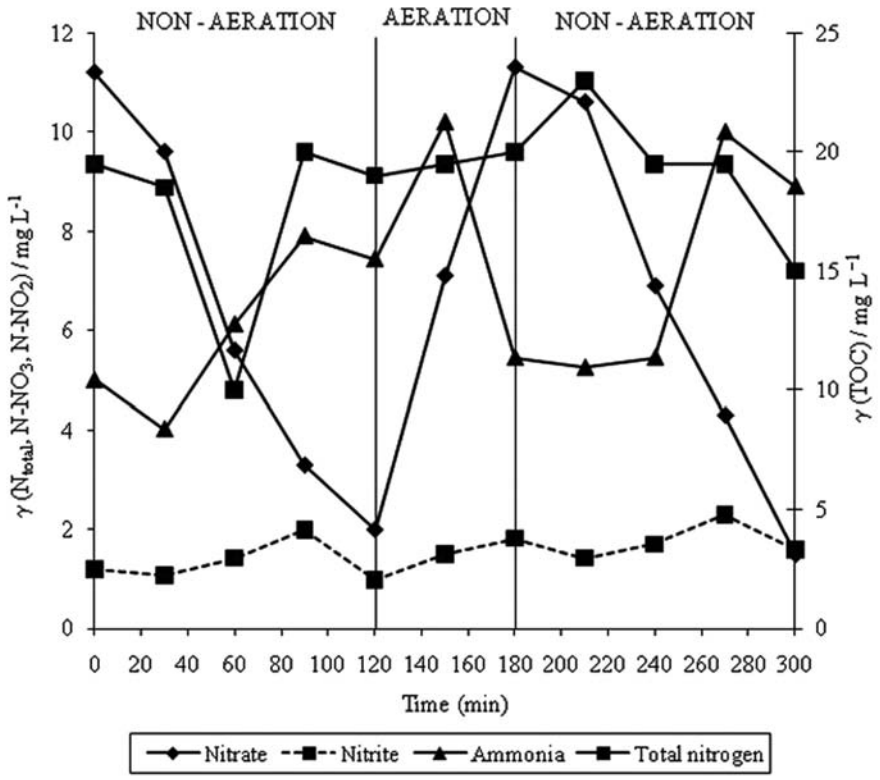


Fig. 7. Continuous treatment of synthetic wastewater containing 0.36 g/L of glucose in MBR 2 (C5) with intermittent aeration (60 minutes aeration and 120 minutes non-aeration).

here as well, nitrate concentration was high, while the ammonia concentration dropped down to 5 mg/L. Since the concentration of ammonia in the incoming water amounted to 60 mg of $N\text{-}NH_4$/L, it can be concluded that the sludge had a good nitrification activity. The concentration of total nitrogen dropped by up to 50% in relation to the concentration in the incoming water, which mounted to 60 mg/L. TOC concentration was low and ranged from 2.2 to 1.2 mg/L, but in the second anaerobic stage there occurred a sharp increase to 8.4 mg/L. At that point, an increase in the concentration of total nitrogen and ammonia occurred, while the concentration of nitrates continued to decrease, but at a lower rate than during the first denitrification, which all points to a decrease of the denitrification rate.

In the experiment (C5), the concentration of glucose in the incoming water increased to 0.36 g/L (Fig. 7). Aeration lasted 60 minutes here as well, and the time without aeration lasted 120 min. The results indicate the good nitrification and denitrification capability of activated sludge. Nitrite concentration was low and did not change significantly. Total nitrogen concentration decreased from 60.3 mg/L (in incoming synthetic water) to 4.02 mg/L in the outgoing water. It is evident that the removal of total nitrogen and ammonia was better with incoming, with a glucose concentration of 0.36 mg/L than with a concentration of 0.18 mg/L, which confirms the observation from the batch denitrification experiment, that the process is significantly stimulated by the concentration of organic substrate, the increase of which stimulates the faster adaptation of heterotrophic microorganisms to nitrate consumption instead of oxygen consumption.

Following that, a third experiment, C6, was conducted, with 0.72 g/L of glucose in synthetic wastewater with the same aeration and non-aeration intervals (60 and 120 min) (Fig. 8). The results indicate the good nitrification and denitrification capability of activated sludge. Nitrite concentration was low and did not exceed 0.17 mg/L. Total nitrogen concentration dropped from 68 mg/L in the incoming water to 2–12 mg/L in the processed water. The best nitrogen removal was observed here. In the second denitrification stage, nitrate concentration dropped to zero, and the glucose concentration in the incoming water was too high to be completely used up in the process of denitrification, so the TOC started to increase, while the concentration of ammonia and total nitrogen also

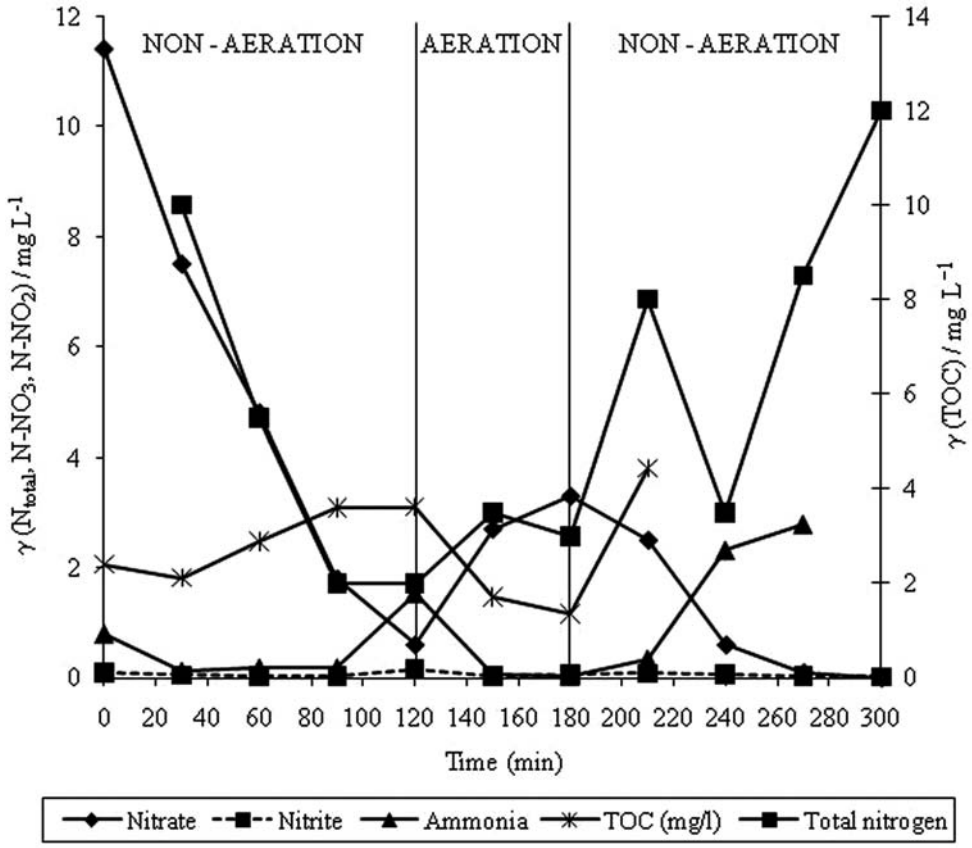


Fig. 8. Continuous treatment of synthetic wastewater containing 0.72 g/L of glucose in MBR 2 (C6) with intermittent aeration (60 minutes aeration and 120 minutes non-aeration).

increased. The C/N ratio in this experiment amounted to 4.125. In the work of Guo et al. [17], with similar concentrations of total nitrogen in incoming water, and with a C/N ratio of 3.5, total nitrogen dropped to less than 2 mg/L. The somewhat greater drop in total nitrogen in the outgoing water, with a lower carbon source concentration in outgoing water, was probably caused by working in a bioreactor with a discontinuous substrate flow, and not in a continuous reactor as in this work.

### 3.4. Specific oxygen uptake rate (SOUR)

Table 7 shows the SOUR of the activated sludge under different conditions of glucose dosage in both MBRs. The initial oxygen concentrations were always about 7 mg/L and their plots over time showed linear decrease. Anaerobic conditions were achieved after 5–10 min after turning off the aeration in MBR 1, and after 18.5–21.5 min in MBR 2. The addition of glucose and the manner of adding did not significantly affect the oxygen uptake rate, although it was expected to. In the work of Rodde-Pellegrin et al. [18] the oxygen consumption in the respiratory activity of activated sludge in an MBR with intermittent aeration depended directly on the nature and the quantity of added substrate.

Table 7
Specific oxygen uptake rate

| | Glucose (g) | SOUR (mg $O_2$/gMLSS h) |
|---|---|---|
| MBR 1 | 0 | 8.53 |
| | 4 | 10.98 |
| MBR 2 | 0 | 5.94 |
| | 10 | 5.67 |

### 3.5. Membrane performance

Membrane performance was monitored by daily pressure measurement and by clean water permeability measurement, which was done before and at the end of the experiment. In order to mitigate membrane fouling in the aeration phase, air bubbles introduced from under the membrane through the diffusers were used in both MBRs, while the prevention of membrane fouling in the non-aeration phase was performed by circulating the activated sludge suspension taken from

the bottom of the bioreactor by a centrifugal pump along the membrane surface. Since the permeate fluxes were rather low (5.5 and 4.3 L $m^{-2}$ $h^{-1}$ for MBRs 1 and 2, respectively) a stabile membrane filtration was achieved throughout the experiments. Transmembrane pressure slowly increased during the prolonged cultivation for the MBR 1 but never exceeded 0.2 bars. Therefore, no chemical cleaning was performed during the experiments, while irregular membrane relaxation occurred when wastewater flow had to be interrupted for maintenance purposes. Permeability of the membrane for clean water for the MBR 1 decreased about 80% during 4 months period of continuous cultivation, while the clean water permeability for the hollow fibre membrane in the MBR 2, which was operated for one month, decreased from 500 to 200 L $m^{-2}$ $h^{-1}$ $bar^{-1}$. Both membranes were successfully cleaned chemically by immersion in hypochlorite solution after the end of the experiments after which they regained original clean water permeability.

## 4. Conclusions

Based on the conducted research, it can be concluded that the mixed culture in the MBR at high SRT developed a good and stable nitrification activity, which successfully converted ammonia into nitrate, with no regards to the alternating aerobic and anaerobic stages of the process. The denitrification stage presented a greater problem, because of the time needed for the microorganisms to adapt from oxygen metabolism to nitrate reduction. An additional carbon source had a positive effect on the rate of denitrification. The addition was better when done at the start of the denitrification stage than in batches during the denitrification or with a continuous carbon supply. During continuous treatment of synthetic wastewater, the best nitrogen removal was achieved with intermittent aeration with 60 minutes of aeration and 120 minutes without aeration, and with the highest glucose concentration in the synthetic wastewater of 0.72 g/L.

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