

# DEAMMONIFICATION PROCESS IN THE MEMBRANE ASSISTED BIOREACTOR

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## ABSTRACT

Conventional ammonia-rich wastewater nitrification/denitrification usually requires a large amount of energy for aeration and an addition of organic carbon, which becomes a major cost factor. In comparison with classical nitrification/denitrification, deammonification is a process which allows reducing both aeration and the addition of external- carbon source to the system. This paper presents the possibility of implementation of the deammonification process into the membrane assisted bioreactor. In one of the reactors partial nitrification was observed while in the other reactor the observed anammox process resulted in 20% nitrogen removal and gas was produced. This paper gives only rough ideas of how the system works.

## KEYWORDS

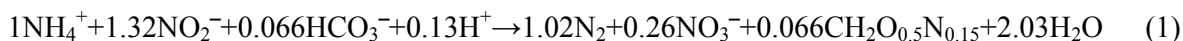
Anammox, deammonification, membrane-assisted bioreactor, nitrogen removal

## INTRODUCTION

Processes of micro – and ultrafiltration become more and more popular in environmental engineering. Membrane-assisted bioreactor (MBR) is a system, which combines activated sludge process with micro - or ultrafiltration membranes. The use of membrane instead of the secondary clarifier in the processes of the wastewater treatment generates a lot of advantages, for example high efficiency of selectivity, which results in good quality of the effluent, retention of all the biomass, which effects in facilitating of the sludge retention time (SRT) control, reliability of the unit operation, easily enhancement of the unit scale, because of module construction and the compact dimensions of the whole system. Apart from these advantages in membrane assisted bioreactor excess sludge production is lower than in conventional activated sludge systems (Ghyoot et al., 2000, Rols et al., 1997), what becomes important on account of the cost connected with treatment of the sludge. COD removal achieves high level and the nitrification of the ammonia nitrogen concentration typical for municipal wastewater is usually complete (Rosenberger et al., 2002, Cicek et al., 2001, Nagaoka et al., 1999).

Also progress in wastewater treatment processes takes place. The deammonification process was discovered by scientists few years ago. The deammonification is a process, that compared to conventional nitrification – denitrification, requires considerably smaller amounts of carbon and oxygen. It is the process, which combine partial nitrification with anaerobic ammonium oxidation (ANAMMOX) process in one single reactor or proceeds as a two-step process. This process allows to reduce aeration and eliminate necessity of external carbon source addition to the system.

In comparison with classical nitrification – denitrification systems, this system allows to reduce aeration and external carbon source addition over 50 and 100% respectively. Moreover, the slower growth speed of the participating micro-organisms makes a considerably lower emergence of surplus sludge (Fux et al., 2002, Seyfried et al., 2001). The Anammox process is an autotrophic process, therefore a complete conversion of ammonia nitrogen to nitrogen gas can take place without any addition of organic carbon. In this process, ammonium is converted to dinitrogen gas with nitrite as an electron acceptor (Strous et al. 1997). Based on mass balances over anammox enrichment cultures, the anammox stoichiometry was estimated to be (Strous et al. 1999b):



The Anammox process is characterized by high capacity (2,6 kg N/m<sup>3</sup> d), therefore it can be used for design compact system for wastewater treatment (van Dongen, 2001).

The overall reaction of the partial nitrification combined with anaerobic ammonium oxidation (ANAMMOX) process is as follows (Sliemers et al., 2002):



By the combining membrane process and deammonification process a new, high efficient system can be created to facilitate high ammonia wastewater treatment.

## **MATERIALS AND METHODS**

The flow scheme of the experimental system is shown in Fig. 1. For purpose of this research two reactors of 36 L were used: MBRA – membrane assisted bioreactor with deammonification process and MBRAN – membrane assisted bioreactor with Anammox process. Reactor for deammonification process (MBRA) was fed by synthetic wastewater, which contained 2 g NH<sub>4</sub>Cl/L, 5 g NaHCO<sub>3</sub>/L, 0.096 g Na<sub>2</sub>HPO<sub>4</sub>/L and 0.1 g of broth/L. Second reactor (MBRAN) was fed by the effluent from the MBRA. The feed was dosing by the peristaltic pump and the same way the permeate was sucked up. In both reactors the temperature was kept above 30 °C. In the MBRA aeration was kept on the level 0.3 – 0.4 mg O<sub>2</sub>/L, only for mixing and partial nitritation. The MBRAN was only mixed.

Configuration of the research system and the operational condition have been changed during experiment period.

In both reactors VA TECH WABAG flat sheet membranes were used. Pore size was 0.4 μm and the total surface area was equal to 0.116 m<sup>2</sup>.

The operation conditions are given in table 1.

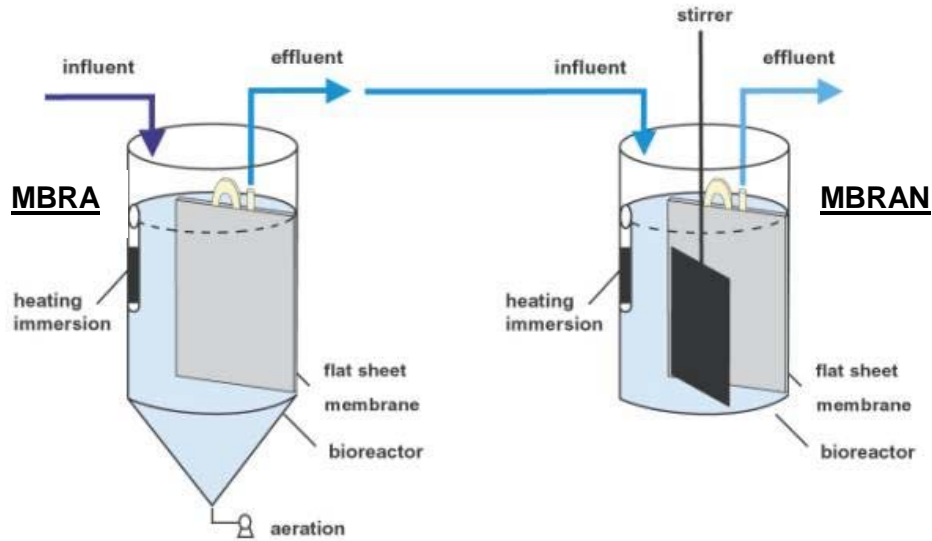


Figure 1. Scheme of the investigated system

Table 1. Operation conditions of the MBRA and MBRAN

PARAMETER	UNIT	VALUE	
		MBRAN	MBRA
Reactor volume	L	36	36
Flow rate	L/d	9	9
Hydraulic retention time (HRT)	d	4	4
COD <sub>0</sub> before 86th day	mgO <sub>2</sub> /L	366 - 616	366 - 616
COD <sub>0</sub> after 86th day	mgO <sub>2</sub> /L	245 - 466	27 - 193
COD <sub>0</sub> after 170th day	mgO <sub>2</sub> /L	0 - 19	21 - 80
NH <sub>4</sub> <sup>+</sup> - N <sub>0</sub> before 86th day	mg/L	151 - 586	151 - 586
NH <sub>4</sub> <sup>+</sup> - N <sub>0</sub> after 86th day	mg/L	461 - 530	465 - 545
NH <sub>4</sub> <sup>+</sup> - N <sub>0</sub> after 170th day	mg/L	3.1 - 281	526 - 567
Sludge organic loading rate before 86th day	g COD/g MLSS d	0,135 - 0,465	0,034 - 0,114
Sludge organic loading rate after 86th day	g COD/g MLSS d	0,037 - 0,112	0,003 - 0,026
Sludge organic loading rate after 170th day	g COD/g MLSS	0 - 0,004	0,002 - 0,008
Sludge NH <sub>4</sub> <sup>+</sup> -N loading rate before 86th day	g NH <sub>4</sub> <sup>+</sup> -N/g MLSS d	0,140 - 0,510	0,042 - 0,124
Sludge NH <sub>4</sub> <sup>+</sup> -N loading rate after 86th day	g NH <sub>4</sub> <sup>+</sup> -N/g MLSS d	0,072 - 0,144	0,047 - 0,084
Sludge NH <sub>4</sub> <sup>+</sup> -N loading rate after 170th day	g NH <sub>4</sub> <sup>+</sup> -N/g MLSS d	0,007 - 0,067	0,026 - 0,070
Biomass concentration before 86th day	g MLSS/L	0,4 - 1,2	0,2 - 2,4
Biomass concentration after 86th day	g MLSS/L	1,3 - 2,4	1,4 - 2,6
Biomass concentration after 170th day	g MLSS/L	1,3 - 2,7	2,2 - 5,5

During the research period the samples were taken from influent, effluent and mixed liquor at least three times a week. The pH was measured by portable WTW pH-meter, dissolved oxygen and temperature by portable WTW DO-meter, biomass concentration, COD was measured by dichromate method, Kjeldahl nitrogen and ammonia nitrogen were determined by means of Kjeltec System 1026 Tecator, nitrite and nitrate nitrogen were determined colorimetrically and respiratory activity of the first and the second stage of the nitrification were measured as described in (Surmacz-Górska et al., 1996). Additionally, according to Anthonisen, the free ammonia and free nitrous acid concentration were calculated (Anthonisen et al., 1976).

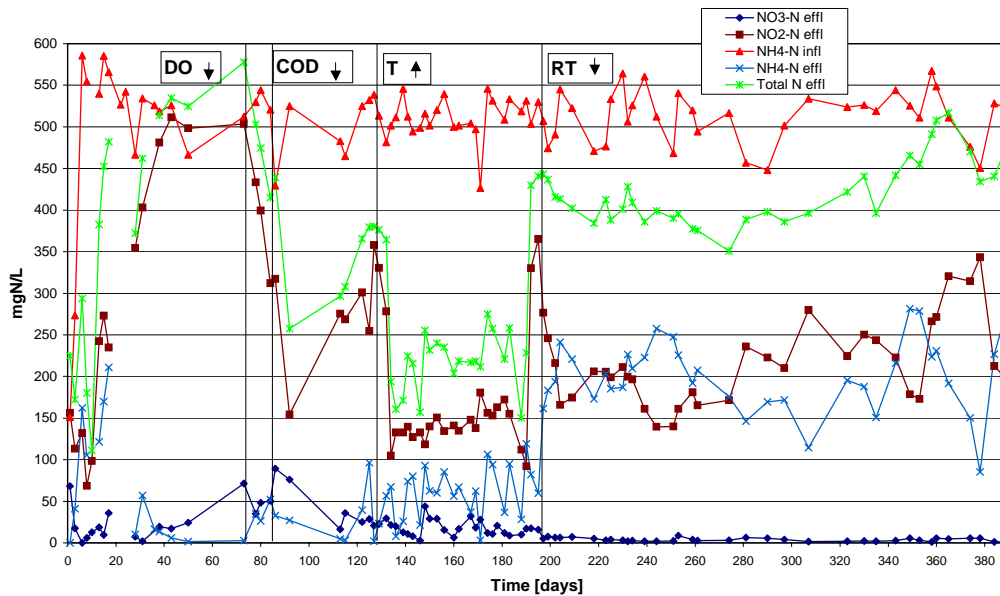
On the 15th, 20th and 29th of May 2003 three batch test were performed. Test were performed in 2 L reactor. Activated sludge from MBRA was stirred for 24 hours without aeration and without feeding in order to remove substrate and starving the sludge. After 24 hours sludge was thickened to 0.2 L and then reactor was filled with feed up to 2 L. The samples were taken in the following intervals: first test – 0, 20, 40, 60 min., 1.5, 2, 3, 4, 6 and 26 hours, the second and third tests – 0, 1, 2, 3, 4 and 5.5 hours. The feeds were composed as described in table 2.

**Table 2.** Composition of the feeds used in the batch tests. Values are in g/L

	FIRST TEST	SECOND TEST	THIRD TEST
NH <sub>4</sub> Cl	-	-	0.38
KNO <sub>3</sub>	0.36	0.25	0.25
NaNO <sub>2</sub>	-	-	-
NaHCO <sub>3</sub>	6.7	6.7	6.7

**RESULTS AND DISCUSSION**

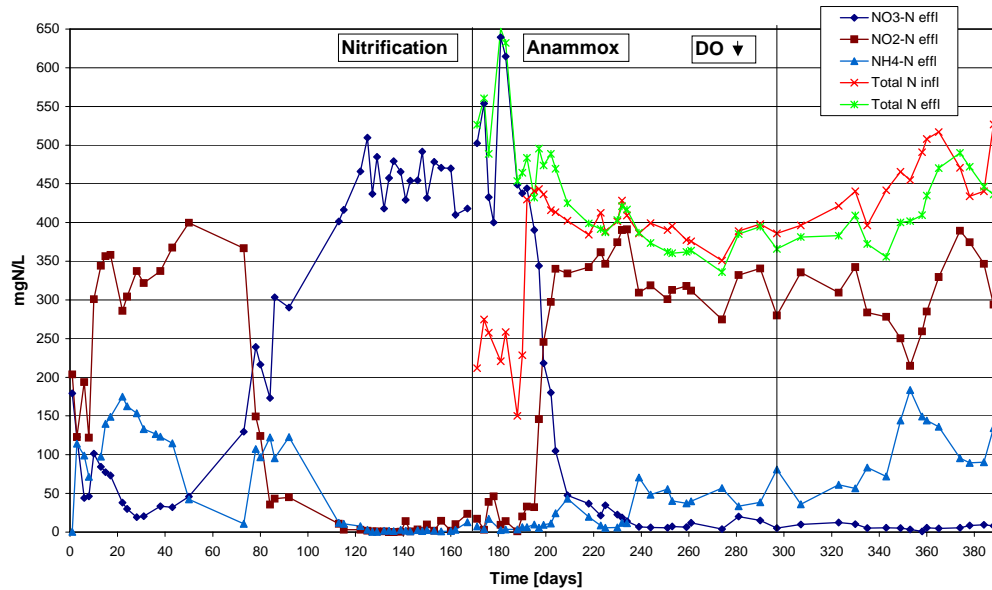
During first 73 days of the experiment nitrification process was carried on in the MBRA. Reactor was fed with the influent characterised by average COD amounted to 486 mg/L, average NH<sub>4</sub><sup>+</sup>-N concentration equal to 495 mg/L and pH corrected to 8.5. Nitrite nitrogen was the main product of ammonia oxidation in this period of the experiment (Fig. 2).



**Figure 2.** Nitrogen conversion in the MBRA.

As the first stage of nitrification was established with high concentration of nitrite nitrogen, parameters have been changed in order to introduce deammonification process. Since 73th day of the experiment dissolved oxygen decreased to the level 0.5 – 0.7 mg O<sub>2</sub>/L, next since 86th day average COD was lowered to 67 mg O<sub>2</sub>/L and since 127th day the temperature was increased above 30 °C (Fig. 2), and the dissolved oxygen was kept at the low level around 0.3 – 0.4 mg O<sub>2</sub>/L. The biocenosis of the MBRA gave very quick answer for the introduced changes. The decrease of dissolved oxygen concentration in the reactor and biodegradable organic carbon in influent resulted in the slight drop of nitrites concentration but neither ammonia nor nitrates increase was observed in the effluent in the same time (Fig. 2). However the most significant effects were observed after the increase of temperature in the reactor. The concentration of nitrite nitrogen dropped drastically below the level of 150 mg NO<sub>2</sub><sup>-</sup>-N/L, nitrate nitrogen was still kept at the low level but ammonia nitrogen increased slightly in effluent not exceeding the concentration of 100 mg NH<sub>4</sub><sup>+</sup>-N/L. Between 134th and 188th day of research, the total nitrogen was removed from 50 to 71%, and the average level of total nitrogen removal in this period was 58%. However, it seems probable that such significant removal of total nitrogen was caused by an accumulation of intermediate forms of nitrogen oxidation, which were not measured. It seems most probable that it was hydroxylamine which is intermediate of ammonium oxidation. Moreover, sudden increase of nitrite nitrogen concentration from 92.2 mg NO<sub>2</sub><sup>-</sup>-N/L in 190th day to 365 mg NO<sub>2</sub><sup>-</sup>-N/L in 195 day of the experiment was observed what can confirm this hypothesis. Due to the fact that nitrite/ammonium ratio in 195th day was unfavourable for Anammox process (it was 6.1:1 in 195th day), the HRT was decreased from 4.2 days to 3.2 days. It caused drop of nitrite concentration and increase of ammonium concentration at the same time. During the period from 202th day to 360th day of the experiment, the average nitrite/ ammonium ratio was 1.05, which was favourable for Anammox process. However it should be mentioned that stoichiometry nitrite/ammonium ratio is equal 1.32:1 according to Eq. (1). The average level of total nitrogen removal in this period was 20.2%. Since 307th day influent flow rate was decreased from 11 L/d to 7 L/d for keeping favourable nitrite/ammonium ratio. After 360th day significant increase of nitrite concentration and decrease of ammonium concentration was observed. At the same time total nitrogen removal decreased. Due to unfavourable nitrite/ammonium ratio, the HRT was changed by increase of influent flow rate from 7 L/d to 9 L/d. It caused improvement nitrite/ammonium ratio and insignificant increase of total nitrogen removal at the end of experiment.

Since 170th day of the experiment the MBRA was joined with the MBRAN in this way that the MBRAN was fed with effluent from the MBRA. During first 170 days in the MBRAN the nitrification of ammonia-rich wastewater with sludge retention time (SRT) of 10 days has been investigated. High ammonia nitrogen removal was observed from the very beginning of the experiment reaching 81%. At the same time nitrite nitrogen was the main product of ammonia oxidation in this period of experiment. Its concentration exceeded 300 mg NO<sub>2</sub><sup>-</sup>-N/L. The second stage of nitrification process was inhibited by high concentration of the free ammonia, which exceeded 62.6 mg/L. After 30 days of the experiment in the MBRAN gradual increase of nitrate nitrogen was observed and since 78th day nitrate nitrogen was the main product of nitrification process exceeding nitrite nitrogen concentration (Fig. 3). Stable ammonia nitrogen removal reaching in average 98% was accompanied by very low ammonia and nitrite nitrogen concentrations in effluent. Almost all ammonia nitrogen was oxidised to nitrate nitrogen, which concentration in effluent levelled at around 450 mg NO<sub>3</sub><sup>-</sup> - N/L. Total nitrogen removal in the MBRAN was low and oscillated around 9% in average (Fig. 3).

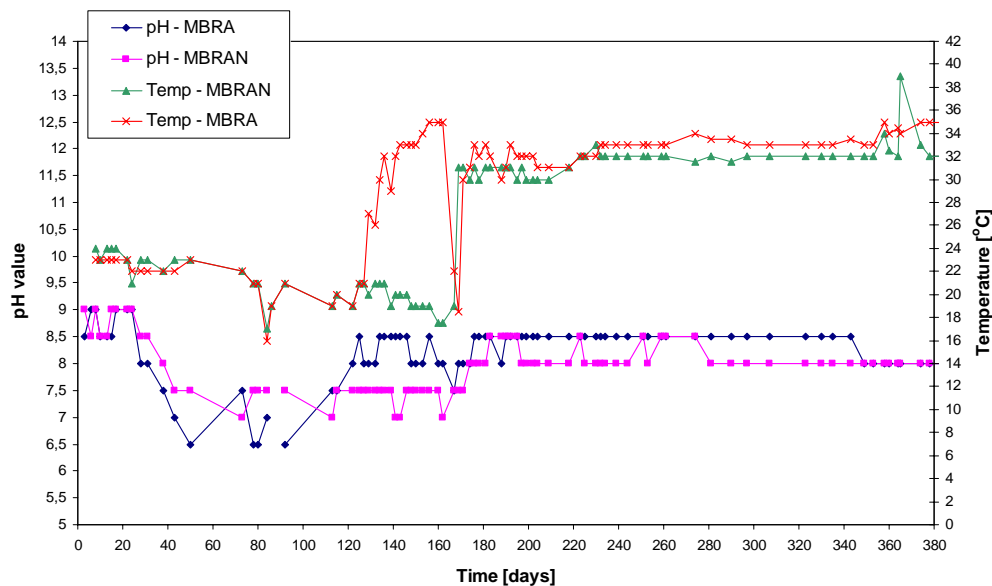


**Figure 3.** Nitrogen conversion in the MBRAN.

Since 170th day the MBRAN was fed with effluent from the MBRA and start up to the Anammox process, which concerns only reaction between nitrite and ammonium. The temperature in the reactor was increased above 30 °C and the dissolved oxygen concentration was decrease to the level 0.3 – 0.4 mg O<sub>2</sub>/L only for sludge mixing. Additionally the aeration system did not offer very efficient oxygen transfer to biomass. After combination of these two reactors, in the MBRAN ammonia and nitrite nitrogen concentrations in effluent was kept at the low level and the nitrate was the main product. Furthermore, total nitrogen concentrations in effluent was considerably higher than in the influent (Fig. 3). It seems probable that such significant difference between effluent and influent was caused by an accumulation of intermediate forms of nitrogen cycles, which were not measured. It seems most probable that it was hydroxylamine, which is intermediate of ammonium oxidation. After 195th day of the research the nitrate concentration dropped drastically to the level of 50 mg NO<sub>3</sub><sup>-</sup>-N/L on 209th day and after 239th day of the experiment it was kept below 10 mg NO<sub>3</sub><sup>-</sup>-N/L. At the same time nitrite concentration began rapidly increase to the level exceeding 300 mg NO<sub>2</sub><sup>-</sup>-N/L (Fig. 3). The average level of nitrite nitrogen in effluent from 204th day to 297th day was 332.3 mg NO<sub>2</sub><sup>-</sup>-N/L. The ammonium concentration was kept between 2.6 and 70.4 mg NH<sub>4</sub><sup>+</sup>-N/L. After 188th day of the experiment total nitrogen concentrations in effluent was insignificant higher than in the influent. It was caused by drastic biomass concentration decrease in the MBRAN. Between 225th and 239th day, total nitrogen concentrations in effluent and in the influent were equal and at the same time biomass concentration was on the same level. After 239th day to 297th day nitrogen removal was observed reaching on average 4.6 % what was the result of bacteria growth. After 197th day of experiment the operation condition of the MBRAN were changed. The volume was increased from 25L to 36L and influent flow rate was decreased to 7 L/d. Moreover the aeration was turned off, and reactor was only stirred. Additionally MBRAN was inoculated with sludge from rotating biological contactor, where anammox process was detected. After these changes, nitrogen removal began insignificantly increase and gas production was observed in the reactor. Total nitrogen removal gradually increased up to 365th day of the experiment when it was rapidly stopped. That could be caused by significant and unexpected temperature increase from 32 °C to 39 °C (Fig. 4) what could inhibit anammox activity. Moreover, unfavourable nitrite/ammonium ratio in effluent from MBRA was observed. On 386th day of the experiment the MBRAN was inoculated again. Moreover reactors were separated and the MBRAN was fed

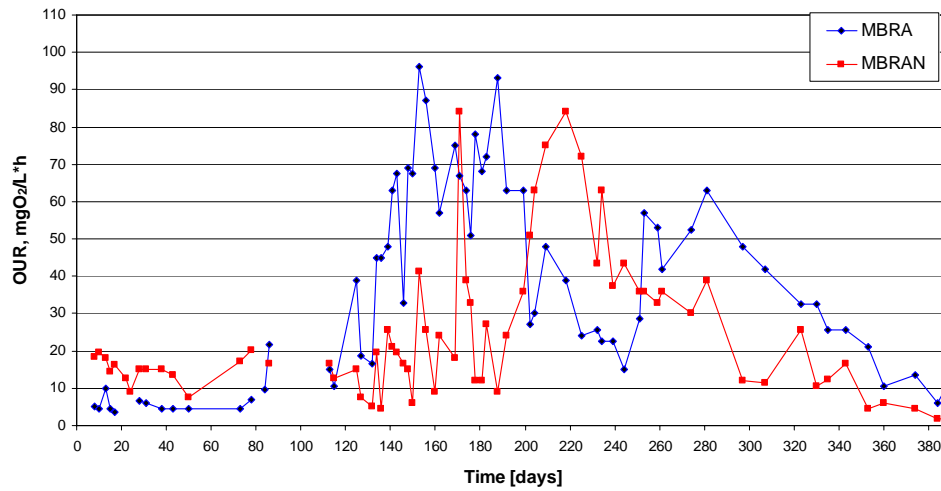
synthetic wastewater, which contained 0.955 g NH<sub>4</sub>Cl/L, 1.28 g NO<sub>2</sub><sup>-</sup>-N/L, 3.5 g NaHCO<sub>3</sub>/L and 0.096 g Na<sub>2</sub>HPO<sub>4</sub>/L. After these changes, significant gas production in the reactor was observed.

The pH values measured in the reactors reflected very well the character of occurring processes. In the influent to the both reactors pH value was corrected and maintained at the stable value of 8.5. In the beginning of the experiments, when both reactors operated in the same way, pH in both reactors dropped to the values 7.5 and 6.5 respectively in the MBRAN and in the MBRA because of dominating first stage of nitrification. In the MBRAN pH value was stable and equal 7.5 till the end of the nitrification process investigation. However in the MBRA, when deammonification process was introduced, pH increased to 8-8.5 (Fig. 4). Since the reactors were joined for introduction of anammox process in the MBRAN, pH increase to 8-8.5 in this reactor was observed. It agrees with theories that within Anammox process ions H<sup>+</sup> produced at ammonia oxidation to nitrites are used in further reactions of nitrite reduction to gaseous forms (Schalk et al., 1998; Siegrist et al., 1998) what confirms that for observed process Anammox bacteria are responsible. In both reactor temperature was kept above 30 °C, after introduction of the studied processes. When the nitrification process was investigated the temperature was kept around 20 °C (Fig. 4).



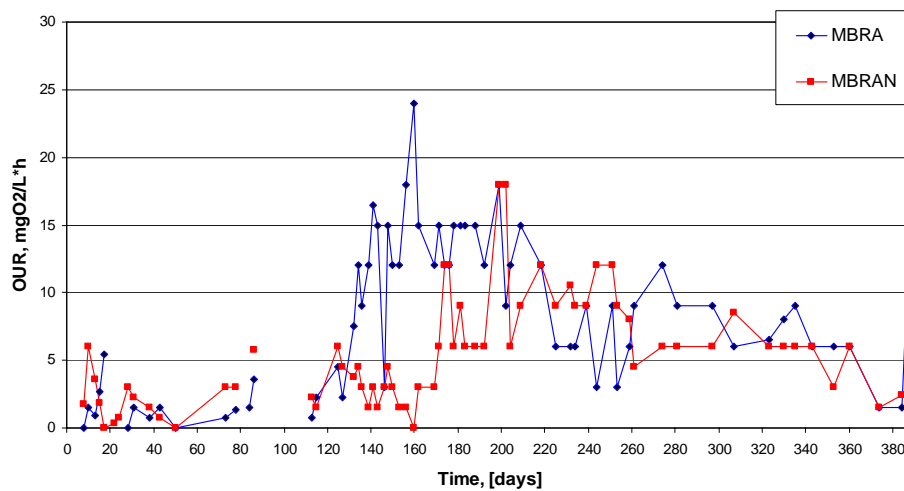
**Figure 4.** Temperature and pH variations in the MBRA and MBRAN

Interesting information about microorganisms activity gave OUR measurements. The activity of Nitrosomonas-like bacteria in both reactors was comparable at the beginning of experiments when the reactors operated in the same way (Fig. 5). Introduced nitrification process resulted in the increase of OUR by Nitrosomonas-like bacteria in the MBRA although the ammonia nitrogen concentration in the influent to the both reactors was the same. Also in the MBRAN, after switching it from nitrification to Anammox condition, significant increase of activity was observed. In both reactor Nitrosomonas-like bacteria activity was being significantly changed. It could be explain by adaptation microorganisms to changed conditions in the reactors. After 218th day and 281th day of the experiment gradual decrease of OUR by Nitrosomonas-like bacteria was observed in the MBRAN and in the MBRA respectively (Fig. 5), although the decrease of the nitrites was not observed. It can be explain by finished bacteria adaptation and lack of oversensitivity.



**Figure 5.** OUR of Nitrosomonas-like bacteria in the MBRA and the MBRAN

Over three-time higher activity of Nitrobacter-like bacteria in the MBRA than in the MBRAN (during the nitrification process investigation) is more difficult to explain (Fig. 6). Probably another group of bacteria have the same vulnerability to sodium chlorate and their activity was measured as the activity of Nitrobacter-like bacteria. In experiments carried out by Lazarova et al. (1999), the respirometric tests showed a high activity of nitrite oxidizers, whereas DNA tests proved very low Nitrobacter content. It is also probable that these bacteria are still present in the reactor but not active. On the other hand, the results of pure experiments carried out by Bock et al. (1988) indicate that Nitrobacter is capable of growing effectively in absence of dissolved oxygen. In order to answer this question more detailed experiments have to be done.



**Figure 6.** OUR of Nitrobacter-like bacteria in the MBRA and the MBRAN

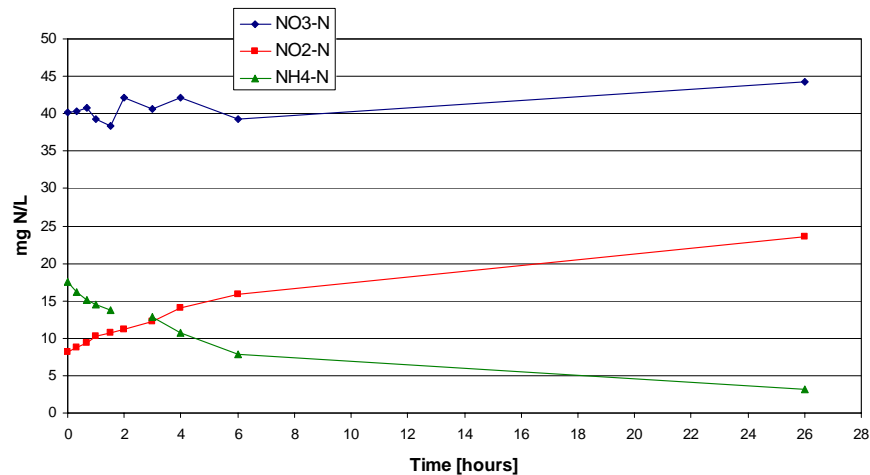
On the 15th, 20th and 29th of May 2003 three batch test were performed. The results of these tests are presented on Fig. 7, 8 and 9 respectively.



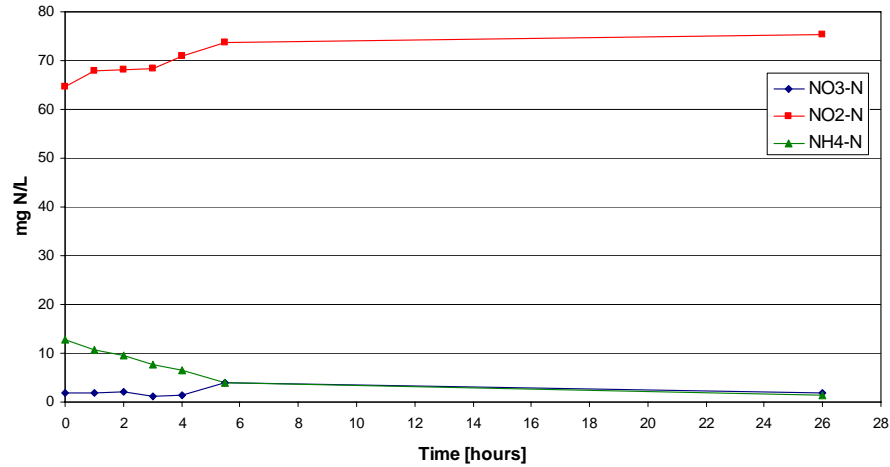
First test was carried out to check if endogenous denitrification took place in the MBRA. At the beginning of test nitrates concentration were 40.2 mg  $\text{NO}_3^-$ -N/L, nitrites concentration were equal 8.2 mg  $\text{NO}_2^-$ -N/L and ammonium was 17.5 mg  $\text{NH}_4^+$ -N/L. During the test ammonium concentration decreased and at the same time increase of nitrites concentration was observed. The nitrates concentration remained at the same level. Also nitrogen removal was not observed. It proved that endogenous denitrification did not take place in the MBRA.

The second test was carried out to check if nitrites were oxidized to nitrates and then nitrates were reduced to nitrites again in the MBRA. It could explain the observed activity of Nitrobacter-like bacteria in the reactor. At the beginning of test nitrates concentration was 1.9 mg  $\text{NO}_3^-$ -N/L, nitrites concentration was equal 64.7 mg  $\text{NO}_2^-$ -N/L and ammonium was 12.7 mg  $\text{NH}_4^+$ -N/L. Like in the first test decrease of ammonium concentration and at the same time increase of nitrites concentration was observed. Also in this test nitrates concentration remained on the same level and nitrogen removal was not observed. It proved that nitrites oxidizing to nitrates and then nitrates reducing did not take place in the MBRA.

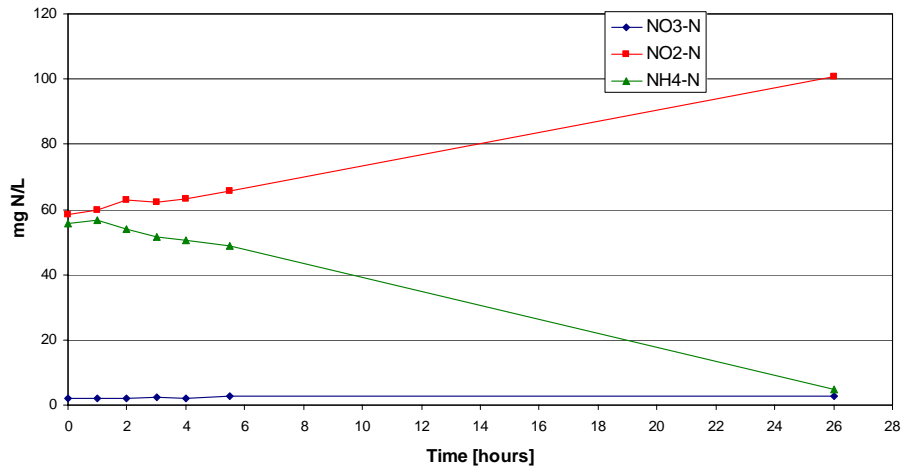
The third test was carried out to check if Anammox process took place in the MBRA. At the beginning of test nitrates concentration was 2.1 mg  $\text{NO}_3^-$ -N/L, nitrites concentration was equal 58.4 mg  $\text{NO}_2^-$ -N/L and ammonium was 55.7 mg  $\text{NH}_4^+$ -N/L. Like in the previous tests decrease of ammonium concentration and at the same time increase of nitrites concentration was observed. Also in this test nitrates concentration remained on the same level. Nitrogen removal was very low, it was 7%, and gas production wasn't observed in the reactor. It follows the Anammox process did not occur in the MBRA.



**Figure 7.** Nitrogen conversion results in test from the 15th of May 2003



**Figure 8.** Nitrogen conversion results in test from the 20th of May 2003



**Figure 9.** Nitrogen conversion results in test from the 29th of May 2003

## CONCLUSIONS

The batch test carried out proved that in spite of favourable conditions (temperature above 30 °C, dissolved oxygen around 0.5 mg O<sub>2</sub>/L, pH between 8-8.5, and nitrite/ammonium ratio around 1), the Anammox process wasn't occurred in the MBRA. On the other hand, when the nitrite/ammonium ratio in this reactor was close to 1, nitrogen removal achieved on average 20.2%. Moreover the Anammox process was observed in the MBRAN, when nitrogen removal increased to 20% and gas production was observed. Such long period of implementation could be explained by very low growth rate of Anammox bacteria. Doubling time is 11 days (van Dongen et al., 2001). Christian Fux reported that doubling time could be even much slower, and amounting 29 day. That results proved possibility for implementation these processes in membrane-assisted bioreactor. It must be said that these studies gave only rough ideas how system behaves. More detailed experiments have to be done.

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