IMPLEMENTATION OF ANAMMOX PROCESS IN THE MEMBRANE ASSISTED BIOREACTOR

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ABSTRACT
This report shows the feasibility of successful start-up of anammox process in the membrane assisted bioreactor. Synthetic wastewaters were used as an influent to the reactor. At the temperature above 30°C, dissolved oxygen concentration below 0.3 mg O₂/L and at the very low contents of biodegradable organic compounds within 5 months of process operation over 75% of nitrogen removal was reached. Nitrite and ammonia nitrogen were below 10 mg/L and very intensive gas production was observed. At the same time nitrate concentration was around 30 mg/L. Additionally two batch test were made to confirm the process.

KEYWORDS
Anammox, deammonification, membrane-assisted bioreactor, nitrogen removal

INTRODUCTION
Membrane assisted bioreactor (MBR) is a system, which couples activated sludge process with the membrane separation of treated effluent from mixed liquor. There are several advantages associated with the MBR, which make it a valuable alternative over other treatment techniques. First of all, the retention of all suspended matter and most soluble compounds within the bioreactor leads to excellent effluent quality, retention of all the biomass, which effects in facilitating of sludge retention time (SRT) control and allows operation at much higher biomass concentration (Cicek 2002, Rosenberger et al. 2002, Gao at al., 2004). Additionally 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. Membrane assisted bioreactor is more compact system than conventional processes, significantly reducing plant footprint (Cicek 2002, Till et al. 2001). The MBR has emerged as an alternative treatment process, especially in cases where space and water resources are limited and high quality product water is required. Industrial wastewater, which is difficult to treat and requires long sludge ages, and wastewater operations where settling and clarification problems are regularly encountered are potential areas of application (Cicek 2002).

Nitrogen removal is an important aspect of wastewater treatment. During the last few years, the deammonification process was discovered and examined by scientists. The deammonification is a process, that compared to conventional nitrification – denitrification, requires considerably smaller amounts of organic carbon and oxygen. It is the process, which combines partial nitrification with anaerobic ammonium oxidation (ANAMMOX) process in one single reactor or proceeds as a two-step process. Anammox is biological process to remove ammonium from wastewater, whereby
under anaerobic conditions ammonium is converted to nitrogen gas with nitrite as electron acceptor (Strous et al. 1997). Hydroxylamine (NH₂OH) and hydrazine (N₂H₄) were identified as intermediates of the Anammox process (Jetten et al. 2001, Schmidt et al. 2002). Anammox bacteria consume ammonia and nitrite in ratio 1:1.3 (Schmidt et al. 2003, Szatkowska et al. 2003). The excess of nitrite is oxidized anaerobically to nitrate. The overall reaction of anammox process is as follows (Strous et al. 1999b, van Dongen et al. 2001)

\[
1\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 2.03\text{H}_2\text{O}
\]

In experiments carried out by Wyffels et al. (2003) ratio for ammonia and nitrite nitrogen was 1:1.43. Anammox process is reversibly inhibited by oxygen and irreversibly by nitrite at concentrations in excess of 70 mg N/L for several days (Fux et al. 2002, Schmidt et al. 2003). The Anammox is an autotrophic process so there is no need for external carbon to support the formation of dinitrogen (Johansson et al. 1998, Strous et al. 1997). Disadvantages of this process is low growth rate of Anammox bacteria, what causes a long start-up period for the Anammox process (van Dongen et al. 2001, Fux et al. 2002).

Due to low growth rate it is necessary to retain all the biomass. Combination membrane and Anammox processes allows to create new high efficient and compact system.

**MATERIALS AND METHODS**

The laboratory reactor for anammox process was operated during 6-month period. The flow scheme of the experimental system is presented in Fig. 1. For purpose of this research, membrane assisted bioreactor of 36 L was used. Reactor was continuously fed by synthetic wastewater, which contained NH₄Cl, NaNO₂, NaHCO₃, Na₂HPO₄ and additionally on the end of experiment 5 – 10% of landfill leachate were added to the synthetic wastewater. The feed was dosing by the peristaltic pump and the same way the permeate was sucked up. The temperature was kept above 30 ºC. The reactor was also equipped with mixer.

Operational condition of the research system and the composition of synthetic wastewater 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 m².

**Table 1. Operation conditions of the MBRA and MBRAN**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>UNIT</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactor volume</td>
<td>L</td>
<td>36</td>
</tr>
<tr>
<td>Flow rate</td>
<td>L/d</td>
<td>9 – 23,5</td>
</tr>
<tr>
<td>Hydraulic retention time (HRT)</td>
<td>d</td>
<td>4 – 1,52</td>
</tr>
<tr>
<td>COD₀</td>
<td>mgO₂/L</td>
<td>4.9 – 60</td>
</tr>
<tr>
<td>NH₄⁺ - N₀</td>
<td>mg/L</td>
<td>17.8 – 74.5</td>
</tr>
<tr>
<td>NO₂⁻ - N₀</td>
<td>mg/L</td>
<td>21.8 – 98.1</td>
</tr>
<tr>
<td>Biomass concentration</td>
<td>g MLSS/L</td>
<td>3.5 – 12.4</td>
</tr>
<tr>
<td>Temperature</td>
<td>ºC</td>
<td>33.8 ± 0.6</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.9 ± 0.1</td>
</tr>
</tbody>
</table>
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 16th and 23rd of March 2004 two batch test were performed. Test were performed in 2 L reactor. Activated sludge from membrane assisted bioreactor 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, 0.5, 2, 4, 7 and 24.5 hours, the second test – 0, 2, 4, 6, 8, 10, 12, 14 and 25 hours. The feeds were composed as described in table 2.

<table>
<thead>
<tr>
<th></th>
<th>FIRST TEST</th>
<th>SECOND TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄Cl</td>
<td>0.1777</td>
<td>0.191</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>0.2957</td>
<td>0.320</td>
</tr>
<tr>
<td>NaHPO₄</td>
<td>0.045</td>
<td>0.045</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>2.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The process was performed at the temperature above 30°C, dissolved oxygen concentration below 0.3 mg O₂/L, average pH amounted to 8 and at the very low contents of biodegradable organic. Ratio for nitrite and ammonium nitrogen in the influent was around 1. According to the literature data, that conditions were favourable for AnaMmox process (van Dongen et al. 2001, Jetten et al. 2002).
At the beginning of the experiment, nitrite nitrogen was the main product of ammonia oxidation. At the same time ammonium and nitrate nitrogen concentration was below 10 mg/L (Fig. 2). During first 30 days of experiment, nitrogen removal decreased from 27 to 10 % and average removal was 19.2% (Fig. 3).

**Figure 2.** Nitrogen conversion during start-up of the Anammox process in the membrane assisted bioreactor.

**Figure 3.** Nitrogen removal and nitrogen removal efficiency in the membrane assisted bioreactor.
It seems most probable that it was caused by high nitrite concentration in the reactor, which was close to toxic value for Anammox bacteria activity. An average nitrite concentration was 64.8 mg \( \text{NO}_2^-\text{-N}/\text{L} \). Fux et al. (2002) reported loss anammox activity when the nitrite concentration remain above 60 mg \( \text{NO}_2^-\text{-N}/\text{L} \). For this reason the total nitrogen concentration in an influent had to be reduced from 100 mg/L to 40 mg/L. After this change, the nitrite concentration dropped significantly to the level 1 to 6 mg/L. Additionally in 40th day the nitrogen removal was 56.8%, however in 47th day of experiment nitrogen removal dropped drastically to 0. At the same time nitrate concentration increased to 38.5 mg \( \text{NO}_3^-\text{-N}/\text{L} \). In order to decrease nitrate concentration the nitrogen load to the reactor was increased to 50 mg N/L. It caused drop of nitrate concentration to 27.7 mg \( \text{NO}_3^-\text{-N}/\text{L} \) and slight increase of nitrite. From 54th to 72nd days of research total nitrogen removal in the reactor was low and oscillated around 13.5% in average (Fig. 3). At the same time nitrate nitrogen was the main product of ammonia oxidation and nitrite and ammonium nitrogen concentration were below 10 mg/L. For the purpose of nitrate nitrogen concentration decrease and on the other hand for nitrogen removal increase the total nitrogen concentration in the influent was gradually increased to the level 102.2 mg N/L. Furthermore, hydraulic retention time (HRT) was gradually decreased from 4 to 2.4 days. Introduced changes, caused drop of nitrate concentration and slight increase of nitrite and ammonia concentration. Total nitrogen removal increased from 4.1% in 72nd day to 42.9% in 86th day. Shortly after these changes, intensive gas production was observed. From 86th to 110th days of research the process was stable and overall efficiency of nitrogen removal was 39.5% (Fig. 3). At the same time nitrite and ammonium nitrogen concentration was on average 14 and 21 mg/L respectively, whereas nitrate concentration gradually decreased form 39.1 mg \( \text{NO}_2^-\text{-N}/\text{L} \) in 83rd day to 17.5 in 110th day (Fig. 2). In order to increase nitrogen removal, in 110th day of experiment, total nitrogen concentration was increased to 133.6 mg N/L on average, and additionally HRT was gradually decreased from 2.4 to 1.5 days. At first introduced changes resulted in the slight drop of nitrogen removal efficiency, however after few days nitrogen removal efficiency was raising again with the maximum value of 63.8% in 131st day of research (Fig. 3). Also nitrite and ammonia concentration, after initial growth, began gradually decrease. Nitrite nitrogen concentration was 24 mg \( \text{NO}_3^-\text{-N}/\text{L} \) on average. Due to the fact that in 131st day nitrite concentration reached 1.6 mg \( \text{NO}_2^-\text{-N}/\text{L} \) and ammonia was 16.6 mg \( \text{NH}_4^+\text{-N}/\text{L} \) (Fig. 2), nitrogen concentration in the influent was increased to 166.4 mg N/L and ammonia to nitrite ratio was changed from 1:1 to 1:1.3, which is favorable for anammox process according to Eq. (1). After these changes nitrogen removal efficiency increased to maximum value of 74.9% in 142nd day of research (Fig. 3), additionally very intensive gas production was observed in the reactor. Nitrite and ammonia nitrogen were below 10 mg/L and very intensive gas production was observed. At the same time nitrate concentration was around 30 mg \( \text{NO}_3^-\text{-N}/\text{L} \). Since then, drastically drop of nitrogen removal was observe, and in 156th day nitrogen removal efficiency decrease to 25.4% and gas production in the reactor was much lower, moreover nitrite nitrogen concentration raised to value of 74 mg \( \text{NO}_2^-\text{-N}/\text{L} \), which is toxic value for anammox activity (Fux et al. 2002, Schmidt et al. 2003). It seems probable that such unexpected breakdown of the anammox process was caused by very intensive growth of algae (Chlorophyta) on the wall of reactor and in the sludge. Due to high nitrite concentration, influent nitrogen load had to be reduced nearly 50% (Fig. 2). Additionally reactor was covered with aluminum foil to protect from the light and algae growth. This changes caused increase of nitrogen removal efficiency to 51%. However the process was very unstable and in 166th day of research, nitrogen removal efficiency dropped to 14.8% and nitrite concentration increased to 38.3 mg \( \text{NO}_2^-\text{-N}/\text{L} \). It seems most probable that process was such unstable because there was lack of mineral elements like K, Fe or Mg in the influent. In order to introduce this mineral elements to the reactor, 5 – 10% of landfill leachate were added to the synthetic wastewater from 168th day of experiment. This caused increased of nitrogen removal, which reached 44% on average (Fig. 3), on the end of research period.
The Anammox process can be characterized by a very high potential capacity equal 2.6 kg N/m³·d (van Dongen et al. 2001). In ours research the highest capacity was much lower (0.071 kg N/m³·d). However, there are some correlation between nitrogen loading rate and nitrogen removal rate. As it is clearly visible on Figure 4a, along with growth of nitrogen loading rate, increase of nitrogen removal rate was also observed. On the one side it was caused by adaptation growth of bacteria responsible for anammox process but on other hand when the nitrogen loading rate was stable between 86th and 110th days of experiment also nitrogen removal rate was stable despite biomass concentration growth was observed (Fig. 4c). When after 110th day the nitrogen loading rate was increased the intensive rise of nitrogen loading rate was observed and also nitrogen removal efficiency has been improved. It seems probable that anammox bacteria work better with higher capacity. Very interesting is also correlation between nitrogen loading rate on the stable level 0.075 – 0.09 kg N/m³·d and nitrogen removal rate (Fig. 4b). In this range, increase of nitrogen removal rate was also observed but the correlation coefficient was very low so it is not a linear correlation. In this case when nitrogen loading rate a bit decreased the nitrogen removal rate was still increasing. I could be caused by bacteria adaptation, and higher participation of anammox bacteria in biomass. On the other hand it is also possible that such big nitrogen loading rate was to high for bacteria activity and it was one reason of break down the process after 142nd days of experiment. For explanation that matter more detailed experiment have to be done.

In the influent pH value was corrected and maintained at the value of 7.8 – 8.1. The pH values measured in the reactor was stable and equal 7.7 – 8.1 (Fig. 4) and was usually equal the value in the influent what agrees with theories (Schalk et al. 1998, Siegrist et al. 1998). Only in 154th day of the experiment, when the anammox process broke down and high nitrite concentration was observed in the reactor (Fig. 2), the pH level dropped from 7.9 to 7.5 what was caused by first stage of nitrification process.
Figure 5. Temperature and pH variation during start-up of anammox process in the membrane assisted bioreactor.

The temperature was kept above 30°C, with an average value of 33.8±0.6°C. Due to the use of thermostats, no significant problems in maintaining the required temperature occurred.

Interesting information about microorganism activity was obtained through OUR measurements. Anammox process is strictly anaerobic, although no pure cultures of anammox bacteria have been grown in the laboratory. Other bacteria are essential to remove one or more toxic products – nitrate, oxygen, organic matter, or free radicals – or they might be required to provide essential nutrients (Mohan et al. 2004). Membrane-assisted bioreactor used for the start-up of anammox process was previously used for nitrification of high ammonia nitrogen concentrations; therefore, it is possible that nitrifiers are still present in the reactor. Measurement of OUR activity of Nitrosomonas and Nitrobacter-like bacteria was much lower than in earlier research (Surmacz-Górska et al. 2003), but these bacteria were still present in the MBR. There was a relationship between OUR Nitrosomonas, Nitrobacter-like bacteria, and nitrite, nitrate concentrations in the reactor. It was especially clear in the 154th day of the experiment, when the anammox process dramatically broke down. At the same time, nitrite concentration increased drastically and also OUR of Nitrosomonas-like bacteria increased. Additionally, from the 154th day of the experiment, an increase in the OUR of Nitrobacter-like bacteria, along with an increase in nitrate concentration, was observed.
On the 16th and 23rd of March 2004 two batch tests were performed. The results of these tests are presented in Fig. 7, 8 respectively.

First test was carried out to check if anammox process took place in the membrane assisted bioreactor. At the beginning of the test nitrates concentration were 4.1 mg NO$_3^-$-N/L, nitrites concentration were equal 59.6 mg NO$_2^-$-N/L and ammonium was 49 mg NH$_4^+$-N/L. During first four hours of the test, ammonium and nitrite concentration decreased and at the same time increase of nitrates concentration was observed. Nitrogen removal was not observed. After 6 hours nitrogen removal efficiency was 5.9%, however after 24.5 hours nitrogen removal increase to 34.5%. At the same time nitrogen removal efficiency in the MBR was 53.7%. Nitrates were the main product of nitrogen oxidation, ammonia concentration was 25.6 mg NH$_4^+$-N/L and nitrites were not detected. It proved that nitrifiers are still present in the reactor and on the other hand also anammox process occur in the membrane assisted bioreactor. During the batch test the reactor was stirred at 76rpm while membrane assisted bioreactor was stirred at 27 rpm. This resulted in higher oxygen concentration during the batch test than in MBR. Dissolved oxygen concentration during the batch test was 0.3 mg O$_2$/L, whereas in MBR was below 0.2 mg O$_2$/L, what could cause less nitrogen removal efficiency. Second batch rest was carried out to confirm the results from the first test. However the mixing of the reactor was changed and reactor was stirrer at 32 rpm what was similar with MBR. At the beginning of the test nitrates concentration were 3.3 mg NO$_3^-$-N/L, nitrites concentration were equal 50.1 mg NO$_2^-$-N/L and ammonium was 51.4 mg NH$_4^+$-N/L. Like in the previous test decrease of ammonium and nitrite concentration and at the same time increase of nitrate concentration was observed. At the few first hours, any nitrogen removal was observed however after 24 hour nitrogen removal efficiency was lower the in first test and it was equal 24%. It seems probable that lower than in the MBR nitrogen removal efficiency and any nitrogen removal during first few hours of the tests was caused by too high nitrite concentration in the reactor, at the beginning of the tests.
Figure 7. Nitrogen conversion results in the test on the 16th of March 2004.

Figure 8. Nitrogen conversion results in the test on the 23rd of March 2004.
CONCLUSIONS
The experiments carried out proved that retention of biomass in membrane-assisted bioreactor and long sludge age give possibility for implementation of anammox process. At the temperature above 30°C, dissolved oxygen concentration below 0.3 mg O₂/L and at the very low contents of biodegradable organic compounds within 5 months of process operation over 75% of nitrogen removal was reached and very intensive gas production was observed. The carried out research confirm that very long time is needed for implementation of anammox process. Doubling time of anammox bacteria is 11 days (van Dongen et al. 2001). Christian Fux reported that doubling time could be even much slower, and amounting 29 day. This research has shown that during the start-up the anammox process is very sensitive and unstable. Probably during the start-up period nitrite concentration in the reactor, which is toxic for anammox bacteria is much lower then 70 mg NO₂⁻/L. More detailed experiments have to be done.

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