Autotrophic nitrogen removal from anaerobic supernatant of Florence’s WWTP digesters

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Abstract

In municipal WWTP with anaerobic sludge digestion, 10–20% of total nitrogen load comes from the return supernatant produced by the final sludge dewatering. In recent years a completely autotrophic nitrogen removal process based on Anammox biomass has been tested in a few European countries, in order to treat anaerobic supernatant and to increase the COD/N ratio in municipal wastewater. This work reports the experimental results of the SHARON-ANAMMOX process application to anaerobic supernatant taken from the urban Florentine area wastewater treatment plant (S. Colombano WWTP). A nitritation lab-scale chemostat (7.4 L) has been started-up seeded with the S. Colombano WWTP nitrifying activated sludge. During the experimental period, nitrite oxidising bacteria wash-out was steadily achieved with a retention time ranging from 1 to 1.5 d at 35°C. The Anammox inoculum sludge was taken from a pilot plant at EAWAG (Zurich). Anammox biomass has been enriched at 33°C with anaerobic supernatant diluted with sodium nitrite solution until reaching a maximum specific nitrogen removal rate of 0.065 kgN kg⁻¹ VSS d⁻¹, which was 11 times higher than the one found in inoculum sludge (0.005 kgN kg⁻¹ VSS d⁻¹). In a lab-scale SBR reactor (4 L), coupled with nitritation bioreactor, specific nitrogen removal rate (doubling time equal to 26 d at 35°C and at nitrite-limiting condition) reached the value of 0.22 kgN kg⁻¹ VSS d⁻¹, which was approximately 44 times larger than the rate measured in the inoculum Anammox sludge.

Keywords

Anaerobic digester; ANAMMOX; nitritation; nitrite; nitrogen removal; SHARON; supernatant

Introduction

In several municipal wastewater treatment plants, the unbalanced ratio of COD/N in the influent wastewater makes it extremely difficult, and not very sustainable, to achieve high heterotrophic denitrification performance and needs to introduce external, readily biodegradable, carbon sources in the anoxic phase. Some of the nitrogen load is due to the return of supernatant from anaerobic sludge dewatering which is an ammonium-rich wastewater (normally 600–1,000 mg L⁻¹ N-NH₄⁺) and does not contain RBCOD: the anaerobic supernatant can constitute 10–20% of the total nitrogen load in the raw influent to WWTPs. A completely autotrophic Sharon-Anammox process (Van Dongen et al., 2001) has been tested in a lab-scale pilot plant in order to remove nitrogen from the anaerobic digester supernatant of S. Colombano WWTP (Florence, Italy) which treats approximately 600,000 p.e. The influent wastewater of this plant is characterised by a low COD/N ratio (6.1 on the average) and a low content of RBCOD (26 mg L⁻¹, 25% of total COD). Co-digesting external organic matter in the S. Colombano anaerobic digesters could increase the nitrogen load of anaerobic supernatant from 10% (current situation) up to 20–30% (Caffaz et al., 2005).

In the SHARON® process (Single reactor high activity ammonia removal over nitrite, Hellings et al., 1998), ammonium is aerobically oxidised to nitrite. NOB wash-out can be achieved in a chemostat at a temperature higher than 30°C by controlling the sludge age...
(SRT, which is equal to HRT in the SHARON process) so that: \( \mu_{\text{max}}(\text{NOB}) < 1/\text{SRT} < \mu_{\text{max}}(\text{AOB}) \). The maximum specific growth rates (\( \mu_{\text{max}} \)) of ammonium oxidising bacteria (AOB) and of NOB depend on temperature, but in a different manner due to their different activation energy (respectively 68 and 44 kJ/mol, Schmidt et al., 2003).

The stoichiometry of the ANAMMOX process (anaerobic ammonium oxidation) is described by the following equation (Jetten et al., 1998; Strous et al., 1998; Van Loosdrecht et al., 2001):

\[
\text{NH}_4^+ + 1.32 \text{NO}_2^- + 0.066 \text{HCO}_3^- + 0.13 \text{H}^+ \rightarrow 1.02 \text{N}_2 + 0.26 \text{NO}_3^- + 0.066 \text{CH}_2\text{O} \text{H}_0.5 \text{N}_0.15^+ + 2.03 \text{H}_2\text{O}
\]

Production of excess sludge is very low, comparable to that of AOB (0.11 gVSS/gN-NH\(_4^+\)), but Anammox microorganisms grow much more slowly than AOB (\( \mu_{\text{max}} \) at 37°C = 0.065 d\(^{-1}\)), so that start-up periods are long. Monod half-saturation coefficients for both ammonium and nitrite are very low (<5 \( \mu \)M, Strous et al., 1999) and this could make this process viable even for ammonium-poor wastewater.

As Figure 1 shows (Fux et al., 2002), the main advantages over conventional nitrification-denitrification are (van Dongen et al., 2001): (1) lower oxygen consumption to nitrification (~57%), (2) no external carbon source is needed for denitrification (~100%), (3) lower production of excess sludge. Operating conditions in an Anammox reactor can greatly affect process efficiency: longer SRTs can reduce nitrite oxidation to nitrate per ammonium removal unit, due to decay, hydrolysis and heterotrophic activity. Thus, total nitrogen removal could be higher than the theoretical stoichiometric value (>89%).

**Materials and methods**

The schematic diagram of the lab-scale nitritation bioreactor (SHARON process) is shown in Figure 2a. The bioreactor was made in Plexiglas, with an external jacket for temperature control. The total reactor volume was 7.4 L, and nitrifying activated sludge from the S. Colombano WWTP was used as inoculum. The pH was controlled by dosing a 4M NaOH solution.

The ANAMMOX biomass enrichment was carried out in a completely mixed bioreactor (30 L) with an external jacket for temperature control, fed in a continuous mode with a peristaltic pump. The supernatant was extracted once a day and the volume of
reactor was variable between 30 and 37 L. The pH control was performed with non-auto-
mated N2-CO2 sparging.

The ANAMMOX reactor (Figure 2b) was a 4-L, PLC-automated SBR and was fed on
effluent from the SHARON process. Nitrogen gas was continuously sparged, while on-line
pH control was ensured by sparging CO2. Oxygen, temperature, pH control and monitoring
of the lab-scale reactor were performed by a biocontroller ADI 1030 Applikon.

In-out samples from all three reactors were taken daily. For N-NO$_2^-$, N-NO$_3^-$, PO$_4^{3-}$
ionic chromatography (DIONEX), flow injection analysis (nitrogen only, ASIA) were
used. N-NH$_4^+$, total COD, soluble COD, total alkalinity, TSS and VSS were measured
according to Standard Methods. Experiments on SHARON-ANAMMOX processes were
carried out on centrifuge centrate (Table 1).

Results and discussion

The SHARON process was operated continuously for 350 d in the pilot plant at
T = 35°C. The experimentation can be divided into three different operating periods as
shown in Figure 3.

In the first period (t = 0–188 d), pH was kept constant (pH$_{\text{const}}$) at first at 7.5 and
then at 7.7. This period is characterised by variable conversion efficiency and by low
nitrate residual concentrations. The retention time was gradually reduced from 4 to 1.5 d,
leading to a partial wash-out of NOB (nitrite oxidising bacteria) and nitrates were
reduced to 4% of the total oxidised nitrogen (N-NOx) in the effluent. Protozoa growth
(100–200 protozoa/mL) in the chemostat reduced the conversion efficiency of AOB
(ammonia oxidising bacteria). Cysts came from the long batch-wise storage (20–30 d) at
ambient temperature of the supernatant (Van Loosdrecht et al., 2001). Growth of proto-
zoa was successfully controlled by using intermittent aeration in 6 cycles/d, each one

Table 1 Characteristics of the centrifuge centrate used in the experiments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>Parameter</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-NH$_4^+$ (mg L$^{-1}$)</td>
<td>737</td>
<td>403</td>
<td>997</td>
<td>%VSS</td>
<td>98.5</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>Alkalinity (mg L$^{-1}$ HCO$_3^-$)</td>
<td>3.324</td>
<td>2.530</td>
<td>4.311</td>
<td>COD$_{\text{tot}}$ (mg L$^{-1}$)</td>
<td>286</td>
<td>119</td>
<td>530</td>
</tr>
<tr>
<td>Alc./N-NH$_4^+$ (mol mol$^{-1}$)</td>
<td>1.17</td>
<td>1.09</td>
<td>1.32</td>
<td>COD$_{\text{sol.}}$ (mg L$^{-1}$)</td>
<td>171</td>
<td>23</td>
<td>328</td>
</tr>
<tr>
<td>N-NO$_2^-$ (mg L$^{-1}$)</td>
<td>0.5</td>
<td>0</td>
<td>2.25</td>
<td>Biodegradable</td>
<td>65</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td>N-NO$_3^-$ (mg L$^{-1}$)</td>
<td>0.05</td>
<td>0</td>
<td>0.2</td>
<td>BOD$_5$ (mg L$^{-1}$)</td>
<td>51</td>
<td>9.5</td>
<td>86.7</td>
</tr>
<tr>
<td>TSS (mg L$^{-1}$)</td>
<td>53.6</td>
<td>3</td>
<td>200</td>
<td>P-PO$_4$ (mg L$^{-1}$)</td>
<td>60</td>
<td>41</td>
<td>92</td>
</tr>
</tbody>
</table>
including 30 min under anoxic conditions. The main results of nitritation at constant pH are shown in Table 2.

In the second period ($t = 189–260$ d), pH was gradually decreased aiming to achieve pH self-regulation conditions and to control the residual nitrate concentration. As pH decreased from 7.7 to 7, the N-NOx/N-NH$_4^+$ ratio decreased from an average value of 6.41 to an average of 2.2.

During the third period ($t = 260–350$ d), the chemostat ran without any external pH control. The SHARON process may run at selfcontrolled pH depending on influent alkalinity consumption. Operating conditions are mainly determined by the molar ratio HCO$_3^-$/NH$_4^+$ in treated wastewater (Hellinga et al., 1999). The mean molar ratio HCO$_3^-$/NH$_4^+$ of the supernatant from anaerobically digested sludge was equal to 1.1 during the whole experimentation. Optimum process conditions were achieved by working at a dilution rate $D = 0.84$ d$^{-1}$ (HRT = 1.19 d), pH = 6.8, residual average alkalinity equal to 41 mg L$^{-1}$ HCO$_3^-$ and total conversion ratio N-NOx/N-NH$_4^+$ equal to 1.22. However, nitrate in the effluent gradually increased to as much as 14% of the total oxidised nitrogen (Figure 3 and Table 3) and it was necessary to increase the dilution rate $D$ to 1 d$^{-1}$ in order to wash NOBs out of the system.

![Figure 3](https://iwaponline.com/wst/article-pdf/53/12/129/432241/129.pdf)

**Figure 3** Time series of effluent ammonium, nitrite, nitrate concentration and of HRT (SHARON process)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± S.D.</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-NH$_4^+$ in (mg L$^{-1}$)</td>
<td>730 ± 123</td>
<td>403</td>
<td>872</td>
</tr>
<tr>
<td>N-NH$_4^+$ out (mg L$^{-1}$)</td>
<td>157 ± 81</td>
<td>4.7</td>
<td>306</td>
</tr>
<tr>
<td>N-NO$_2^-$ out (mg L$^{-1}$)</td>
<td>546 ± 129</td>
<td>361</td>
<td>817</td>
</tr>
<tr>
<td>N-NO$_3^-$ out (mg L$^{-1}$)</td>
<td>24 ± 20</td>
<td>0</td>
<td>83</td>
</tr>
<tr>
<td>Conversion efficiency (%)</td>
<td>77.4 ± 11</td>
<td>56</td>
<td>99.9</td>
</tr>
<tr>
<td>Specific conversion rate (kgN d$^{-1}$ m$^{-3}$)</td>
<td>381 ± 72</td>
<td>111</td>
<td>550</td>
</tr>
<tr>
<td>HCO$_3^-$ in (mg L$^{-1}$)</td>
<td>3,741 ± 293</td>
<td>2,530</td>
<td>4,311</td>
</tr>
<tr>
<td>HCO$_3^-$ out (mg L$^{-1}$)</td>
<td>386 ± 146</td>
<td>54.4</td>
<td>782.7</td>
</tr>
<tr>
<td>4M NaOH solution (mL d$^{-1}$ consumed)</td>
<td>43.3 ± 20</td>
<td>0</td>
<td>70.8</td>
</tr>
<tr>
<td>Dilution rate, $D = 1/$SRT = 1/HRT (d$^{-1}$)</td>
<td>0.68 ± 0.06</td>
<td>0.24</td>
<td>0.85</td>
</tr>
<tr>
<td>COD$_{biomassa}$ out (mg L$^{-1}$)</td>
<td>250 ± 96</td>
<td>93</td>
<td>445</td>
</tr>
</tbody>
</table>
This produced a temporary reduction of nitritation efficiency. At $0.7 < D < 1 \, \text{d}^{-1}$, NOB growth could depend on: (1) gradual acclimation of NOB species at high free-NH$_3$ concentration; (2) lower influence of protozoans on the process, as they could be controlled by intermittent aeration. Experimental results showed that ammonium conversion efficiency decreased from 56 to 50%, as $D$ increased from 0.85 to 0.95 (pH 6.8) to 0.95 (pH = 7.2–7.3, residual alkalinity = 130 mg L$^{-1}$ HCO$_3$).

The Anammox biomass enrichment was carried out (350 d) in a completely mixed bioreactor, 30-L volume, at $T = 33^\circ$C and pH in the range of 7.5–8.3. An Anammox biomass inoculum was taken from a pilot plant at EAWAG (Zurich; Fux et al., 2002). The initial activity was very low (5 gN kgVSS$^{-1}$ d$^{-1}$) and the biomass growth-rate was increased by dosing a synthetic solution composed of anaerobic supernatant diluted with sodium nitrite solution at increasing concentrations. During the first 194 d, the specific nitrogen removal rate increased 11 times (from 5.6 to 62.6 gN kgVSS$^{-1}$ d$^{-1}$, Table 4 and Figure 4), and doubling time was estimated to be around 35 d at $T = 33^\circ$C. The trends of the ratios of N-NO$_2^-$/N-NH$_4^+$ removed and N-NO$_2^-$/N-NH$_4^+$ produced/N-NH$_4^+$ removed progressively approached the stoichiometric coefficients (1.32 and 0.26, respectively), due to Anammox biomass prevailing over other active biomass (Figure 5). The total nitrogen removal efficiency decreased as nitrate increased.

The Anammox process was run (300 d) in a more controlled SBR reactor, which was seeded with enriched Anammox sludge taken from the 30-L bioreactor. The specific activity immediately increased around 1.5 times due to the strict anoxic conditions, online pH control (constant value of 7.8) and higher temperature (35°C). Anammox minimum doubling time was equal to 26d, under nitrite-limiting conditions. Table 5 shows the main results of the experimentation, split into four phases, according to influent wastewater characteristics, as nitrogen removal efficiency (79–86%) was dependent on feed composition. When the heterotrophic activity is high (due to higher COD content), the nitrate production decreases, increasing the overall nitrogen removal efficiency (I and III phases). Conversely, the unbalanced ratio N-NO$_2^-$/N-NH$_4^+$ in the influent, necessary to avoid possible inhibition by nitrite, produced ammonium build-up and decreased the nitrogen removal efficiency (IV phase).

### Table 3 Nitritation (SHARON process): main results during the second period (189–260 d) at self-controlled pH (mean ± S.D.) and $T = 35^\circ$C

<table>
<thead>
<tr>
<th>N-NH$_4^+$ (mg L$^{-1}$)</th>
<th>N-NO$_2^-$ (mg L$^{-1}$)</th>
<th>N-NH$_4^+$ (mg L$^{-1}$)</th>
<th>Conv. NH$_4^+$ (%)</th>
<th>Removal rate (kgN/d/m$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>398.3 ± 98.4</td>
<td>360.9 ± 57.5</td>
<td>56.2 ± 38</td>
<td>50.4 ± 4.2</td>
<td>0.374 ± 0.037</td>
</tr>
<tr>
<td>NO$_2^-$/NH$_4^+$ (mol/mol)</td>
<td>NO$_2^-$/NH$_4^+$ (mol/mol)</td>
<td>D(d$^{-1}$)</td>
<td>N-NH$_4^+$ (mg L$^{-1}$)</td>
<td>COD biomass out (mg L$^{-1}$)</td>
</tr>
<tr>
<td>0.92 ± 0.146</td>
<td>1.06 ± 0.136</td>
<td>0.9 ± 0.093</td>
<td>818 ± 57.6</td>
<td>209.4 ± 75.11</td>
</tr>
</tbody>
</table>

### Table 4 Anammox process: results in the 30-litre bioreactor fed on anaerobic supernatant ($T = 33^\circ$C)

<table>
<thead>
<tr>
<th>30-L bioreactor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental days</td>
</tr>
<tr>
<td>Feed</td>
</tr>
<tr>
<td>N-NO$_2^-$ / N-NH$_4^+$</td>
</tr>
<tr>
<td>N-NO$_2^-$ / N-NH$_4^+$</td>
</tr>
<tr>
<td>N-NH$_4^+$ removal rate (mgN L$^{-1}$ d$^{-1}$)</td>
</tr>
<tr>
<td>N-NO$_2^-$ removal rate (mgN L$^{-1}$ d$^{-1}$)</td>
</tr>
<tr>
<td>Nitrogen removal rate (mgN L$^{-1}$ d$^{-1}$)</td>
</tr>
<tr>
<td>Specific removal rate (mgN g$^{-1}$ VSS d$^{-1}$)</td>
</tr>
</tbody>
</table>
Figure 6 shows the volume-specific removal rate (mg N L\(^{-1}\) d\(^{-1}\)) concerning the three nitrogen species (production of nitrate) during the whole experimentation. The maximum volume-specific nitrogen removal rate reached in the SBR was equal to 0.37 kg N m\(^{-3}\) d\(^{-1}\), while the maximum biomass-specific nitrogen removal rate was equal to 0.22 kg N kg\(^{-1}\) VSS d\(^{-1}\), which was approximately 44 times larger than the rate measured in the inoculum Anammox sludge. The net yield observed was equal to \(Y_n = 0.074 \text{ mg COD/mg } \text{N-NH}_4^+\) (\(Y_{max} = 0.16\)). Figure 7 shows the MLVSS growth together with nitrogen load in the SBR. Anammox activity decreased dramatically and suddenly in both reactors, respectively, by 90% in the 30-L reactor after 200 d, data not shown in this work (Caffaz, 2004), and by 50% in the SBR after 169 days. Then, it slowly recovered with a low growth rate, close to that of the first enrichment period. As a matter of fact, other examples of loss of Anammox biomass activity when fed with anaerobic supernatants can be found in the
scientific literature (Fux et al., 2002). In the inhibition tests conducted by Strous et al. (1999), before the activity decreased, the stoichiometry of the reaction changed, as N-N\textsubscript{2}O\textsubscript{2}/N-NH\textsubscript{4}$^+$ removal ratio increased. Similarly, during the experiments described here, the loss of Anammox activity occurred after an increase of nitrite demand per ammonium unit removed (the N-N\textsubscript{2}O\textsubscript{2}/N-NH\textsubscript{4}$^+$ removal ratio increased from 1.3 to 1.5).

In the 30-L reactor, the sudden loss in the anammox activity occurred when nitrite concentration and ammonium concentration built up, to 40 mgN-N\textsubscript{2}O\textsubscript{2} L\textsuperscript{-1} and to 13 mgN-NH\textsubscript{4}$^+$ L\textsuperscript{-1}, respectively. The loss in Anammox activity in the lab-scale SBR could be due to nitrite accumulation during the SBR feed phase, which may have occurred because of a rapid increase of nitrogen loading rate (Strous et al., 1999), but did not depend on nitrite accumulation only, since nitrite concentration often increased to as high as 15 mg L\textsuperscript{-1} N-N\textsubscript{2}O\textsubscript{2} under ammonium-limiting conditions (N-NH\textsubscript{4}$^+$ = 0) and this did not cause any reduction of Anammox activity. When the nitrite concentration increased due to unbalanced N-N\textsubscript{2}O\textsubscript{2}/N-NH\textsubscript{4}$^+$ ratio in the influent, no problems occurred in the SBR, but when the nitrite concentration increased because of too high nitrogen

<table>
<thead>
<tr>
<th>Table 5 Anammox process: results in the 5-L SBR (T = 35 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I phase</td>
</tr>
<tr>
<td>Experimental days</td>
</tr>
<tr>
<td>Feed</td>
</tr>
<tr>
<td>N-N\textsubscript{2}O\textsubscript{2}/N-NH\textsubscript{4}$^+$</td>
</tr>
<tr>
<td>N-N\textsubscript{2}O\textsubscript{2} removal rate (mgN L\textsuperscript{-1} d\textsuperscript{-1})</td>
</tr>
<tr>
<td>N-N\textsubscript{2}O\textsubscript{2} removal rate (mgN L\textsuperscript{-1} d\textsuperscript{-1})</td>
</tr>
<tr>
<td>N-N\textsubscript{2}O\textsubscript{2} removal rate (mgN L\textsuperscript{-1} d\textsuperscript{-1})</td>
</tr>
<tr>
<td>N-N\textsubscript{2}O\textsubscript{2} removal rate (mgN L\textsuperscript{-1} d\textsuperscript{-1})</td>
</tr>
<tr>
<td>Specific rem. rate (mgN g\textsuperscript{-1}VSS d\textsuperscript{-1})</td>
</tr>
<tr>
<td>% N-NH\textsubscript{4}$^+$ removed</td>
</tr>
<tr>
<td>% N-N\textsubscript{2}O\textsubscript{2} removed</td>
</tr>
<tr>
<td>% N removed</td>
</tr>
<tr>
<td>MLVSS (g L\textsuperscript{-1}, mean)</td>
</tr>
</tbody>
</table>

Figure 6 N-NH\textsubscript{4}$^+$, N-N\textsubscript{2}O\textsubscript{2} removal rate and N-N\textsubscript{2}O\textsubscript{3} production rate in Anammox SBR
loading rate, rapid loss in the Anammox activity occurred. Another possible cause might have been a too rapid increase of soluble phosphate concentration at values > 2 mmol, which could inhibit Anammox biomass (Van de Graaf et al., 1995, 1997), coupled to the increase of nitrogen loading rate. Under steady-state conditions (NO$_2^-$ limiting, constant nitrogen load) a sudden reduction of activity never occurred.

**Conclusions**

The SHARON-Anammox process was selected as the best system for the removal of nitrogen from ammonium-rich wastewaters, such as anaerobic supernatants for the low energy consumption and the low excess sludge produced.

The completely autotrophic process was tested in two lab-scale pilot plants fed on anaerobic digester supernatant. Nitritation (SHARON process) was performed in a chemostat at temperature equal to 35°C. The process had a variable conversion efficiency due to protozoa predation (increasing decay coefficient of autotrophic biomass). In pH-controlled conditions, with external feed of alkalinity, an average conversion (N-NH$_4^+$ into N-NO$_2^-$) efficiency of 80% was obtained at HRT = 1.5 d. Under variable-pH conditions (with consumption of the influent alkalinity) it was difficult to maintain a constant ratio of N-NO$_2^-$/N-NH$_4^+$ due to variable nitrogen load, growth of NOB at low dilution rates and protozoa predation.

A stable and high-rate Anammox process was achieved under strictly controlled conditions (constant pH value of 7.8, temperature of 35°C). Anammox minimum doubling time was equal to 26 d, under nitrite-limiting conditions with a maximum value of specific removal rate as high as 220 gN kg$^{-1}$VSS d$^{-1}$.

However, the Anammox biomass presented fragility when exposed to variable nitrogen loads and, possibly, to phosphorus concentrations higher than 2 mmol. The low growth rate and this fragility could jeopardize the start-up and the long-term stability of a real-scale plant.

**Acknowledgements**

We would like to thank engineers Nicola Monami, Valentina Camici, Letizia Lombardi for their care and attention in carrying out the experiments. Thanks also to Publiacqua S.p.A. and their laboratory staff for co-financing, but also for their hospitality and invaluable help.
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